The Journal of Parasitology

Volume 24 JUNE, 1938 Number 3

STUDIES ON *DIROFILARIA IMMITIS* LEIDY, WITH SPECIAL REFERENCE TO THE SUSCEPTIBILITY OF SOME MINNESOTA SPECIES OF MOSQUITOES TO THE INFECTION

CHIA-HSIEN YEN*

Following the discovery that filariasis is transmitted by mosquitoes, it was generally believed that all kinds of mosquitoes had the power to act as suitable intermediate hosts to the filarial parasite. Observations as well as experiments have indicated that this is not true. A species of mosquitoes susceptible in one place may behave quite differently in another. Therefore, the problem of susceptibility is one which must be solved locally. In view of the fact that apart from the studies of S. M. K. Hu at Baltimore, Maryland, no such work had been done in the northern United States, a series of experiments was carried out to determine the susceptibility of some Minnesota species of mosquitoes to infection by *Dirofilaria immitis* Leidy.

METHODS

In conducting the experimental work for the determination of the intermediate hosts of *Dirofilaria immitis* the investigator may be forced to examine a large number of mosquitoes. An abundant supply was bred in the laboratory.

A heavy mortality was always encountered in the first few days after emergence. As various workers have found wild-caught mosquitoes more hardy than bred-out specimens, adult mosquitoes were collected around breeding places. They were caught with a suction tube and released into a transporting cage which was constructed of wire gauze fitted over a rectangular wooden frame, with a sleeve on one side. If not of immediate use for the experiment, they were kept out of the direct sunlight and in a cool place. If necessary, humidity was maintained by covering the top of the cage with moistened cotton.

The transmission experiments were conducted in a wire screened cage 20 by 20 by 26 inches in size. At one end of the cage was a sliding

Received for publication, August 7, 1937.

* The writer wishes to express his deep appreciation to Dr. W. A. Riley, under whose direction the study was conducted. To Dr. A. C. Chandler the writer also wishes to extend his grateful thanks for supplying an infected dog.

door for the entrance of the dog and on top of it a hole, closed by a slide, just large enough for the insertion of the arm for releasing and catching the mosquitoes. The board was removable for cleaning.

Adult mosquitoes to be used for the experiment were deprived of food and moisture for a day to stimulate their appetite for the infective blood meal. They were enclosed during the night with the dog which was muzzled in order to protect the mosquitoes. To insure that the mosquitoes had an ample opportunity to feed on the infective blood the experiments were conducted in the evening for a period of three hours from 8 to 11 P.M. In case the mosquitoes did not feed on the dog readily they were left with it in the cage overnight. Those found to be engorged with blood were isolated into screened lamp chimneys over Petri dishes containing pads of moistened cellucotton. When the blood meal was digested, fresh soaked raisins were put on the screened top of the lamp chimney for food. The mosquitoes found dead during each day were dissected and examined for the filarial larvae. After the infective filarial larvae had been found in the mosquitoes, the remaining specimens of the same lot were etherized and dissected within the next few days.

In dissection, a slit was made on either side of the eighth abdominal segment of the mosquito and the intestinal tract was drawn out posteriorly in a drop of saline solution on a slide. At the earlier stages of infection, the intestinal tract and the malpighian tubules were examined separately with a compound microscope in saline solution on a slide for the filarial larvae which might have succeeded in migrating into the the malpighian tubules and those which might have failed. With mosquitoes that were in a late stage of infection, the head, thorax, and abdomen of the specimen were cut apart in three drops of saline solution on another slide, in order to trace the location of the infective filarial larvae that had broken out from the malpighian tubules.

EXPERIMENTAL STUDY ON THE SUSCEPTIBILITY OF LOCAL SPECIES OF MOSQUITOES TO INFECTION BY *D. immitis*

The assumption that once the microfilariae have been taken up by a mosquito and have passed from the alimentary tract to the tissues they are capable of becoming infective in a given mosquito species is unwarranted. It has often led to the inclusion in lists of effective carriers of many species which, in a strict sense, are really not suitable hosts. Therefore, in the present investigation care was taken to trace the development in every species of mosquitoes tested. Only when the filarial larvae had arrived at maturity, that is, with the infective stage in the mosquito, was it considered as a carrier.

In considering the percentage of infection in the different species of mosquitoes, only those specimens in which all the filarial larvae were

found to be soundly established and well on their way of reaching the infective stage were considered as showing infection.

The intensity of infection was measured by the number of filariae that were able to establish themselves in the host. Only those cases will be considered in this connection in which all the filarial larvae were well established and nearly mature.

Experiments with Aedes stimulans *Walker*

Aedes stimulans has not previously been experimentally tested for its susceptibility to the infection with *Dirofilaria immitis*. A series of experiments was made at various times from June to August, 1936. Altogether 170 mosquitoes of this species were fed on the infected dog. Most of them died before the incubation period of the parasite because of the hot and dry weather prevailing at that time. Of these 170 fed mosquitoes only 21 lived over 10 days. It was found that 10 out of these 21 mosquitoes, or 47 per cent, were positive. In 7 of those 10 mosquitoes the infective larvae were found in the malpighian tubules, and in the remaining 3 they were found both in the malpighian tubules and abdominal cavity. None of the mosquitoes showed any infective larvae that had migrated into either the thorax or the head. The average intensity of infection per mosquito was 8, the maximum 21.

During the course of dissection definite retardation of growth of the filarial larvae was observed in 8 mosquitoes and pigmental encapsulation in 20.

In 6 of the 8 specimens containing the filarial larvae of retarded development, live microfilariae were found in the malpighian tubules on dissection although these specimens lived 5 to 7 days after the infective meal and the other mosquitoes of the same batch contained mostly the typical sausage forms. In one specimen dissected on the 10th day after infection one early pre-infective form was found in one of the malpighian tubules while 4 of the other matured larvae had pierced through the tubules into the abdominal cavity. In another specimen dissected on the 11th day, one dead filaria of the microfilarial stage at the junction of the malpighian tubules and the stomach and 3 live pre-infective forms in the tubules were found along with 3 live infective forms.

Of the 20 mosquitoes in which pigmental encapsulation took place, 12 had the filarial larvae encapsulated in the microfilarial stage, 4 in the sausage stage and 4 partially in the microfilarial stage and partially in the sausage stage. There was no case which showed the pigmental encapsulation when the filarial larvae had developed beyond the sausage stage. It was also found that in mosquitoes which showed the phenomenon of pigmental encapsulation there were still some of the vigorous filarial larvae which were able to escape from being caught. Since the filarial

larvae had established themselves, or were approaching the infective stage in about half of the mosquitoes which lived over 10 days, *Aedes stimulans* may be counted as a susceptible intermediate host of *D. immitis*. On account of its wide distribution and vicious habit it should be regarded as a dangerous potential carrier of the infection.

Experiments with Aedes cinereus *Meigen*

Aedes cinereus, likewise, was tested for the first time to susceptibility to infection with *Dirofilaria immitis*. Altogether 18 mosquitoes of this species were fed on the filariated blood of the dog in the period from June to August, 1936. Of this number 17 were found positive. But only 5 specimens lived over 11 days. In three specimens which lived to the 14th day, the infective larvae had already migrated from the malpighian tubules to the abdominal cavity and thorax. The maximum number of parasites which were found mature in this species of mosquito was 19 and the minimum 2, with an average intensity of infection of 8 per mosquito.

Pigmental encapsulation was encountered in only one specimen dissected on the 5th day after the infective meal. Two out of six sausage forms were completely encapsulated. All other mosquitoes showed their parasites normal in development.

This species, commonly encountered in wooded regions, feeds at any time. Although not particularly abundant, it might be of considerable importance in this state, in view of its high susceptibility to the infection.

Experiments with Aedes canadensis *Theobald*

The susceptibility of *Aedes canadensis,* first reported by Hu in this country in 1931, is now confirmed by the finding of the infective larvae in the labium.

Of 21 mosquitoes of this species which were fed on the infected dog during the latter part of June and the early part of July, 1936, 20 were found positive for the parasite at various periods of incubation. Of 8 which lived over the necessary period of incubation of the parasite, all showed quite heavy infection. The minimum number of parasites per mosquito was 6 and the maximum 22, with an average intensity of infection of 10. Specimens living to the 13th day showed infective larvae in their labia in two out of five cases; on the 14th day, all.

Retardation of growth of the parasite was shown in two specimens dissected on the 5th and 6th day respectively. About half of the parasites in both cases were still in the microfilarial stage while the rest of them had already developed to the typical sausage stage.

Pigmental encapsulation was found in five specimens. The parasites were all encapsulated in the microfilarial stage. The earliest period at which this appeared was about two days. It seems that once the para-

sites succeed in passing this critical period they will likely establish themelves in this species.

Judging from the 8 mosquitoes which lived over the incubation of the parasite *Aedes canadensis* is a very susceptible species, at least under our laboratory conditions. Here again we are dealing with a widely distributed and very abundant mosquito.

Experiments with Aedes vexans *Meigen*

Aedes vexans was first reported by Grassi and Noè and later by Hu to be susceptible to the infection with *Dirofilaria immitis*. According to the latter (1931), this species is a fairly good carrier of the parasite. Of 56 exposed mosquitoes of this species, 45, or 80 per cent, were found to be positive. The average intensity of 10 specimens which lived to the time when matured larvae were found was 10.

In the writer's experiments, *A. vexans* showed even a higher degree of susceptibility than had been reported. Thirteen experiments were carried out during the late part of June, July, August, and early September, 1936. On account of the hot and dry weather of June and July the early experiments were not very successful; practically all the mosquitoes died in the first few days after infection. However, comparatively few of those which fed on the filariated blood of the dog in August and September died early.

Of 129 mosquitoes that were fed throughout the experiments 25 survived to the period when the filarial larvae had completed their development. Every one of these was found positive, so that a 100 per cent infection was obtained for this species. Although the number of survivals was small, it is clear that this species is very susceptible. Among those which died before the required period of incubation of the parasite none was found to be negative. The larvae found in them appeared normal and active. It is most likely that the parasite would have developed to the infective stage if the mosquitoes had lived long enough to allow time for it.

The heaviest infection of the 25 specimens harboring the active matured larvae was 76 and the lowest 2. The average of all counts, the intensity of infection per mosquito, was 37. One specimen dissected on the 12th day showed two infective larvae already in the head. The earliest time when they were found in the labium was 13 days. In one specimen the labium contained as many as 4 infective larvae and the head of the same specimen contained 15. Many specimens were so heavily infected that as soon as they were cut apart in a drop of saline solution a whole mass of parasites could be seen coming out. On seeing such specimens, one is struck by the potentiality of infections if they should be crushed on the skin of susceptible animals.

Pigmental encapsulation was encountered in quite a number of specimens. In 12 cases larvae were found to be encapsulated at the microfilarial stage, in 3 cases at the sausage stage, in 1 case at both the microfilarial and sausage stage, in 2 cases at the pre-infective stage, in 4 cases at the infective stage, and in 1 case at both the pre-infective and infective stages. Thus it seems apparent that the filarial larvae could be caught by pigmental encapsulation at any of their successive stages of development. However, what evidence there is goes to indicate that this species is a very good carrier of *D. immitis*.

Aedes vexans is one of the most widely distributed and abundant mosquitoes in the state. They feed in the shade during the day and are specially annoying at dusk. They not infrequently enter houses freely in search of food. Therefore, this species is, by all odds, an important mosquito in the state.

Experiments with Aedes trivittatus *Coquillett*

Altogether five experiments were carried out with *Aedes trivittatus* in August and September, 1936. Of 16 that had fed on the infected dog none was found to be positive. No trace of infection could be made out from the malpighian tubules. Therefore, the filarial larvae in this species of mosquito must have either been digested in the stomach or eliminated from the anus.

Experiments with Culex tarsalis *Coquillett*

Culex tarsalis is a rather common species which has not previously been tested as to its susceptibility to the infection with *Dirofilaria immitis*. Ten experiments were carried out during the months June, July and August, 1936. Of 49 mosquitoes fed on the filariated blood of the dog, 38 survived to the period when definite development of the larvae could be detected. Thirteen out of the 38 were positive, thus making the total percentage of infection 34.

All the filarial larvae developed normally, but the number which were found to reach maturity was small. The maximum intensity of infection was only 9, the minimum was 1. The average intensity of infection per mosquito was only 3. In no case was more than one infective larva found in the labium, although there might have been five or six larvae in other parts of the body.

Judging from the percentage as well as the intensity of infection, *Culex tarsalis* is certainly not a better carrier than most of the *Aedes*, although no pigmental encapsulation or retardation of growth were observed. The light infection of this species is most probably due to the elimination of most of the filarial larvae before they could establish themselves in the malpighian tubules. But its importance in the spread of the

disease cannot be ignored just because of the low percentage and intensity of infection.

Experiments with Culex territans *Walker*

Six experiments were carried out in July and August, 1936. Altogether 42 mosquitoes were fed on the filaria-infected dog. Of these, 23 survived the required period of incubation of the parasite. Only 5 were then found to be positive.

The intensity of infection was also low in this species. The maximum intensity was 10, the lowest 1. The average intensity of the five mosquitoes harboring the infective larvae was 5. They first appeared in the labium on the 13th day.

This mosquito showed therefore the same degree of susceptibility as *Culex tarsalis* and is not a good carrier of *Dirofilaria immitis* at least under our laboratory conditions. But mention must be made that we are here dealing with a species which has a fondness for entering houses.

The susceptibility of *Culex territans* was reported by Hu (1931). According to him, 9 out of 41, or 22 per cent infected mosquitoes were found to be positive. The average intensity of infection was only 3. The results of the writer's experiments agree with his findings.

Experiments with Culex pipiens *Linnaeus*

Culex pipiens is the common house mosquito of eastern North America. It breeds primarily in rain barrels, cisterns, tanks and catch basins about urban communities. In Minnesota it has been observed to hibernate during the winter in large numbers in the large mushroom caves on the Mississippi River in St. Paul as well as in bee cellars.

Mosquitoes used in the experimental study were collected from the mushroom caves. Several thousands were collected during the period from October to December, 1936. When they were taken to the laboratory they were kept in screened cages covered with moist cellucotton to assure a high humidity of 80 to 90 per cent. In order to avoid loss by sudden change in temperature and to assure the return to activity, these hibernating mosquitoes were subjected first to a 5° C cabinet for a day and then to the 10, 15, 20, 25 and 30° C cabinets at every two days interval. As it was later found that if humidity was kept high, only few mosquitoes would die when they were directly transferred from the cool mushroom cave to the laboratory where the temperature was heated up to about 25° C, mosquitoes of later batches were left in cages covered with moist cellucotton in the laboratory for a week or so to allow them to digest their stored fat. In spite of all the trouble and care in keeping a continuous supply of mosquitoes, only a few of them were induced to feed on the dog, only 28 out of several hundred being engorged. They

were kept in the laboratory at around 25° C to allow the parasites to complete their development. Of these 28 mosquitoes, 7 died in the first two days and 21 lived long enough for a definite diagnosis. Only three of the 21 were found positive. The maximum intensity of infection was 6, the minimum 1, with an average of 3 per mosquito.

Although the susceptibility of this species is low, it must be counted as an important carrier of the disease because of the fact that it is primarily a domestic species, breeding about the urban communities.

With *Culex pipiens,* Hu (1931) found a percentage of infection of 27 and an average intensity of infection of 2.

Experiments with Theobaldia inornata *Williston*

Theobaldia inornata was for the first time tested. Of the 15 mosquitoes of this species that had fed on the filariated blood of the dog during May and June, 1936, 12 were found positive for the parasite when dissected on various days of incubation of the parasite. The larvae recovered from these positive cases were all of the microfilarial stage although they succeeded in migrating into the malpighian tubules. Even in specimens which lived over the incubation period of the parasite only microfilariae were present. In one mosquito which was allowed to live to the 17th day in order to give the parasite ample time for development, one encapsulated microfilaria was found.

The development of larvae in this species seemed to be inhibited by the formation of a pigmental coat around the parasite. It was found that mosquitoes living over 24 hours after the infective meal had almost all of their parasites encapsulated in the brownish black case. Only in one specimen which was dissected on the 12th day of infection was found a dead but unencapsulated microfilaria in one of the malpighian tubules.

As the microfilarae of *Dirofilaria immitis* were found not to be able to undergo development in this mosquito under the experimental conditions, *Theobaldia inornata* is not likely to be a carrier of the heart-worm disease of the dog.

Experiments with Taeniorhynchus perturbans *Walker*

Taeniorhynchus perturbans is another species first tested for susceptibility to the infection with *D. immitis.* Altogether 9 experiments were carried out from July to September, 1936. In this mosquito the filarial larvae showed no development at all. Of 8 mosquitoes which the writer induced to feed on the filaria-infected dog, only one which was dissected on the third day of incubation of the parasite harbored 13 microfilariae in its malpighian tubules, of which 12 were dead and 1 was encapsulated in the pigmental case. All negative specimens showed no sign even of infection of the malpighian tubules, which appeared quite normal after

staining. The larvae in the majority of the mosquitoes must have been either digested in the stomach or eliminated from the anus as is the case is *Aedes trivittatus*.

Experiments with Anopheles punctipennis *Say*

Anopheles punctipennis was reported to be a carrier of *Dirofilaria immitis* by Hu (1931), who stated that every specimen of 35 mosquitoes experimentally infected with *D. immitis* was positive and the average intensity of infection of the 10 specimens harboring the matured larvae was 23.

We carried out four experiments with this species in July, August and September, 1936. On account of the high mortality of the larvae in artificial breeding jars only few mosquitoes were, however, available for experimental study. The total number of mosquitoes that were fed on the infected dog was only 10, of which 7 survived the period for the complete development of the parasite. Every specimen of these was positive. Those which died early also showed heavy infection. One specimen dissected on the 12th day of infection already showed the infective larvae in the labium. The maximum intensity of infection was 37 and the minimum 16. The average intensity of infection was 24 per mosquito. The results agree fully with those obtained by Hu and show that *Anopheles punctipennis* is a very good carrier of *Dirofilaria immitis*.

Experiments with Anopheles maculipennis *Meigen*

Because of the same difficulties as encountered in rearing *Anopheles punctipennis* from the larval stage to the adult, few mosquitoes were available for the experimental study. In two experiments carried out in August, 1936, only 7 mosquitoes were fed. Except two specimens which died on the 5th day of infection the rest lived very long. Every one of them was heavily infected. The maximum intensity of infection was 30, the minimum 8, the average 21. Infective larvae first appeared in the labium on the 13th day of infection.

Judging from its percentage and intensity of infection, *Anopheles maculipennis* is probably about on the same footing with *A. punctipennis* in respect to susceptibility to infection with this parasite. Its susceptibility was first reported by Grassi and Noè (1900). Its importance in the spread of the disease would be great in some regions.

Experiments with other species of mosquitoes

Because of the small number of mosquitoes available for examination, the development of *Dirofilaria immitis* was not traced to its final stage in the following species of mosquitoes: *Aedes spencerii* Theobald, *Aedes excrucians* Walker, *Aedes fitchii* Felt and Young, *Aedes flavescens* Muller, and *Aedes triseriatus* Say.

In both *A. spencerii* and *A. triseriatus* only a single specimen was fed on the filariated blood of the dog. In the specimen of *A. spencerii* which was dissected on the 5th day after infection, 13 sausage-stage larvae were found. At the foci of infection the malpighian tubules showed a definitely swollen condition. The pathological appearance of the tubules of *A. triseriatus* was also very clear but only 7 live sausage-stage larvae were found. It is probable that the parasites in both species can reach the infective stage if enough time is given for their development.

In *Aedes flavescens* two specimens were fed on the infected dog. The specimen which was dissected on the first day after infection contained 85 live microfilariae which had already established themselves in the malpighian tubules, and the other specimen which was dissected on the 7th day after infection contained 44 late sausage-stage larvae already able to wriggle around very actively. Judging from the data obtained for this species as well as for the most of the members of this genus, it seems most probable that *A. flavescens* is a good carrier of *Dirofilaria immitis*.

Six specimens of *Aedes excrucians* were fed on the infected dog. The specimen which lived to the 10th day after infection was negative, but the other five mosquitoes which were dissected on the 6th or 7th day after infection contained live sausage-stage larvae. Of *Aedes fitchii*, five specimens were fed. One contained microfilariae in its malpighian tubules when it was dissected on the 2nd day after infection and two specimens contained sausage-stage larvae when they were dissected on the 5th day. Some encapsulated microfilariae were also observed in one of the two specimens which lived to the 5th day after infection. Of the two specimens which were dissected on the 9th day after infection one gave a negative result and the other contained five pre-infective larvae. The result thus far indicates that both *A. excrucians* and *A. fitchii* are probably in the same class with their near relative *A. stimulans*, as to susceptibility to infection with *Dirofilaria immitis*.

As other species of *Aedes* tested proved to be fairly good carriers of *D. immitis*, it seems very probable that *A. spencerii*, *A. excrucians*, *A. fitchii*, *A. flavescens* and *A. triseriatus* will likewise prove so.

ESCAPE OF FILARIAL LARVAE FROM THE LABIUM OF THE MOSQUITO

When the mosquito began to feed, the labellae moved back and forth simultaneously to locate the place for the insertion of the stylets. At the same time each labella expanded sidewise a little by the simultaneous expansion of the membraneous area which extends obliquely across the labella from the outer margin to the tip. As the insect began an insertion of the stylets, the body was lowered anteriorly and the basal part of the labium bent back like the elbow of an arm. When the terminal labellae

were firmly applied to the surface of the skin, the "Dutton's membrane" was somewhat stretched.

Mosquitoes used for observation on the points of exit of the parasite were those in which the larvae had already entered the labium. This may be easily detected by first etherizing the mosquito and then examining the specimen under a compound microscope. The mosquito recovers in two or three minutes if it is not over-etherized. It was found that the larvae moved vigorously in the labium. Sometimes even three or four larvae were seen in a single labium. They could advance or retreat within the labium without much difficulty. Very often they were found to push out the membraneous tip of the labella by constant insertion of the head against the membrane. The larvae thus broke through the tip of the labella very easily, but it is apparent that once the stylets of the mosquito sank into the tissue and the labellae were applied to the surface of the skin of an animal as was observed during the feeding experiments, the "Dutton's membrane" would probably be the better place of exit, due to its stretching. The larvae were observed to escape from this point more often than from the tip of the labella when the mosquito was artificially fed on a drop of water under a cover slip on a slide.

TISSUE CHANGES IN THE INFECTED MOSQUITO

The most visible changes of the tissues caused by the parasitism of the larvae of *Dirofilaria immitis* in the mosquito, occur in the malpighian tubules. Sections were made to study the histopathology.

In normal condition, the malpighian tubules are tubular bodies with caecal ends, which open into the hind gut. The cells are extremely large. Each cell contains a large nucleus and many large granules. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately, so that a zigzag appearance is given in section. The inner portion of each cell is striated, the lumen being thus rounded by a striated area. The cells themselves are wrapped by a layer of thin peritoneal membrane. A large number of tracheae and tracheal end cells are found in the walls of these tubules.

When mosquitoes were infected with the larvae of *Dirofilaria immitis* the microfilariae were first seen to penetrate and lie within the tissue cells of the tubules instead of in the central lumen. The intercellular membrane of many cells was broken by the active penetration of the larvae. The cells began to enlarge somewhat so that the tubules swelled out at the focus of infection. Later, cells disintegrated, leaving only the peritoneal membrane and the inner cell membrane, but the nuclei remained distinct. Because of the breaking down of the intercellular membrane and distortion of the cells, they were no longer distinct from one another but looked like irregularly shaped and polynucleate cells. When the

larvae reached the infective stage, the cells were very much flattened out and were finally entirely broken down, so that the larvae were only wrapped around by a thin layer of the peritoneal membrane which could be broken through very easily. By this time the larvae could be seen to wander around in the body cavity or among the fat bodies. However, they were generally found to move forward and crowded themselves among the fat bodies in the lower half of the thorax. Only occasionally were they found to lie in the upper half of the thorax. Due to the active movement of the larvae the fat bodies were very much distorted. From the thorax they continued to migrate along the ventral side of the esophagus to the head. The neck region was sometimes packed with them. Muscle fibers were not seen to be much harmed. Even when the larvae wandered into the muscles, they were seen to lie among the muscular bundles parallel to the fibers.

PIGMENTAL ENCAPSULATION OF THE PARASITE

References to the formation of a chitinous capsule around the parasite in mosquitoes are numerous in literature. Worms as well as protozoa, hyphomycetes and bacilli, have been seen encapsulated in this way. The terms adopted by the authors for the structure are variable.

In filariasis Bahr (1912) and Manson-Bahr (1921) described larvae of *Wuchereria bancrofti* in *Aedes variegatus* enclosed in chitinous capsules, like mummies in their coffins. According to this author, dead worms were most frequently isolated in this way. The same phenomenon was observed by Fülleborn (1929) in the malpighian tubules of *Aedes fasciata* infected with the larvae of *Filaria repens*. This author shared the view of Manson-Bahr. Apparently the same structures were observed by Noè (1900) in the malpighian tubules of mosquitoes infected by the larvae of *Dirofilaria immitis* and were considered by him to be products of degeneration ("brown degeneration") of the worms. According to Sambon (1902) the brown capsules seen by Noè were secondary parasites of the worm (comparable to the pebrine of silkworm). It was suggested that they may account for the limitation in the distribution of the parasite.

As to the true nature of these structures, Brug (1916) first considered them to be chitinous corpuscles formed by the mosquitoes as a reaction to the parasites' presence. The suggestion that they are chitinous structures was supported by Welch (1921) and Mayne (1929) in their researches on the "black spores" of Ross in malaria-transmitting mosquitoes. The latter author laid particular stress on the relationship of the "black spores" to the tracheal tubes of mosquitoes. Knowles and Basu (1933) thought it is the rule in insects for foreign bodies or dead parasites within the insects' tissues to become invested with layers

of chitin laid down on them by the chitinogenous cells lining the inner surface of the fine tracheal ramifications.

In the present study a considerable number of filarial larvae were found unable to complete their development in various species of mosquitoes. The young larvae succumbed to the reaction of the host either shortly after migrating into the malpighian tubules, or possibly in some cases even after migrating into the body cavity. The antagonistic action of the tissue of the host was manifest by the accumulation of brownish-black materials about the unwelcome guests. In order to study the formation and nature of the capsule, sections of mosquitoes were also prepared. It was found that the capsules were formed as early as 48 hours after the mosquito was infected. They took no haematoxylin stain. In some sections of mosquitoes early after infection, where the microfilariae were still in the blood content of the stomach, the microfilariae were already encapsulated in the brownish-black capsules. The capsules were structureless and were quite thick and brittle.

The parasites were encapsulated in definite stages. Mostly they were either encapsulated in the microfilarial or the sausage stage. Only rarely were they found in the pre-infective stage. In some cases, even the infective larvae might carry on their body a few brownish-black pieces of this material. From the sections of mosquitoes studied, it seems to me, although more substantial microchemical test is needed, that this material is merely the shed skin of the parasite. Just as is often observed in the bodies of other animals when foreign substances are introduced, an immunity reaction develops and the filarial larvae may fail to shed their skins. The gathering of the colored material on the surface of the skin results in the formation of a rather substantial capsule. Empty capsules of the larval shape were also seen in the sections.

There are no indications that the capsule is connected with the tracheal tubes. In all cases encapsulated larvae are found to lie within the cells of the tubules, being separated from the tracheal endings by the peritoneal membrane. That the capsules are not of chitinous nature is further supported by the findings of the encapsulated larvae in the blood content of the stomach in mosquitoes of an early infection. Therefore the capsules are only products of degeneration.

Some mosquitoes showed many microfilariae in the hind gut. This may account for the fact that the number of larvae reaching maturity in a given species of mosquito is always much less than that ingested. Probably many of them are eliminated in the first few days. It may also account for some of the non-susceptibility of certain species of mosquitoes which showed no sign of infection even when fed on a heavily infected dog. Encapsulated larvae were also seen to be passed out from the hind gut.

CONCLUSIONS

As *Dirofilaria immitis* is a noxious parasite of dogs, its larvae are apparently also injurious to the intermediate hosts, mosquitoes. The mosquitoes develop a certain resistance to infection, which varies with the species concerned. In *Aedes trivittatus* the larvae were killed very early in the stomach; in *Theobaldia inornata* and *Taeniorhynchus perturbans,* although they might succeed in migrating into the malpighian tubules, they were destroyed there without any development; in other species tested they completed their development, the percentage and intensity of infection being quite variable. The question of why some species of mosquitoes are more suitable as the intermediate hosts than others appears to be unexplainable by the morphological and taxonomical differences of mosquitoes, for even members of the same species vary to a considerable degree in their susceptibility to infection with the parasite. What mechanism is involved in bringing about these variations in host susceptibility of the different species of culicid hosts is difficult to determine until we have a better understanding of the physiology and pathology of the insect.

Twelve of the seventeen local species of mosquito in Minnesota, which the writer has tested, may be placed in three classes, according to the degree of fitness as intermediate host, or, the degree of susceptibility to infection with the parasite, as follows:

Class I. Mosquitoes in which larvae do not develop at all, that is, species entirely unsuitable as intermediate hosts.

Group 1. Species in which larvae are unable to migrate into the malpighian tubules and either die in the gut after a short time or are eliminated through the anus: *Aedes trivittatus.*

Group 2. Species in which larvae are able to migrate into the malpighian tubules but soon die there: *Theobaldia inornata* and *Taeniorhynchus perturbans.*

Class II. Mosquitoes in which some larvae complete their development and some die as a result of pigmental encapsulation.

Group 3. Species in which some larvae die of capsule formation in the microfilarial (early embryonic) stage: *Aedes canadensis.*

Group 4. Species in which some larvae die of capsule formation either in the microfilarial stage (embryonic stage) or in the sausage stage (first larval stage): *Aedes stimulans* and *Aedes cinereus.*

Group 5. Species in which some larvae die of capsule formation either in the microfilarial stage (embryonic stage), in the sausage stage (first larval stage), or in the pre-infective stage (second larval stage): *Aedes vexans.*

Class III. Mosquitoes in which no encapsulation is noted but filarial larvae complete their development normally.

Group 6. Species in which a comparatively small number of larvae complete their development, that is, species having a low intensity of infection: *Culex territans, Culex tarsalis* and *Culex pipiens.*

Group 7. Species in which a great many larvae complete their development, that is, species having a high intensity of infection: *Anopheles maculipennis* and *Anopheles punctipennis.*

It is by no means necessary that species classified under Class II must be less susceptible than those under Class III, for it is quite possible that in spite of the pigmental encapsulation of some of the filarial larvae there are still a large number completing their development. The degree of susceptibility can only be measured by the percentage as well as the intensity of infection. A species of mosquitoes having a low percentage of infection but a high intensity may be just as efficient a carrier as a species of mosquitoes having a high percentage of infection but a low intensity.

As the filarial larvae can develop in each of these species, they are all susceptible. In both *Anopheles maculipennis* and *A. punctipennis* every specimen fed showed a positive infection. The intensity of infection in the two species was high, being more than 20 per mosquito. In respect to its susceptibility, *Aedes vexans* was even higher than the two species of *Anopheles.* It had not only a 100 per cent infection but also an intensity of infection of around 40 per mosquito. *Aedes stimulans, A. cinereus* and *A. canadensis* were less susceptible because the average intensity of infection for each of them was around 10. Nevertheless, the former two species had also a very high percentage of infection of around 100. In *Culex territans* and *C. tarsalis* the percentage of infection was 21 and 34 respectively. The average intensity of infection was 5 and 3 respectively. The susceptibility of these two species was somewhat the same. *Culex pipiens* had a percentage of infection of 14 and the average intensity of infection of 3. Among these nine species which have been proved to be susceptible, *Aedes vexans, Anopheles maculipennis* and *A. punctipennis* were the most susceptible; *Aedes stimulans, A. cinereus* and *A. canadensis* were less susceptible; and *Culex territans, C. tarsalis* and *C. pipiens* were the least susceptible.

It seems apparent that the remaining five species, namely, *Aedes spencerii, A. excrucians, A. fitchii, A. flavescens* and *A. triseriatus* in which the filarial larvae were not traced to their final stage of development, are susceptible although no definite conclusion can be drawn as to degree. The evidence indicates that they are to be grouped with *Aedes stimulans, A. cinereus,* and *A. canadensis,* having a comparatively low intensity of infection.

Thus there are in Minnesota quite a few of the common species of mosquitoes experimentally proved to be the carriers of *Dirofilaria immitis.* But their mere presence is no indication that they must be of importance in the local spread of filariasis in dogs.

The factors which determine the propagation of filariasis in dogs are very complicated and may be summarized in general as follows:

(1) Infected dogs with a sufficient number of filarial embryos in their peripheral blood must be accessible to and bitten by susceptible mosquitoes.

(2) Susceptible mosquitoes must be present in sufficient density within the range in which the infected dogs are present.

(3) Conditions of temperature and humidity must exist which favor the development of the parasites in mosquitoes and the survival of the mosquitoes.

(4) Dogs must finally be accessible to and bitten by the surviving infected mosquitoes.

These requirements cannot be satisfied in all places, and consequently canine filariasis is not a disease which geographically is co-extensive with the dog distribution, nor is it co-extensive with the mosquito distribution. But it is always possible that in areas where lack of certain of the foregoing factors account for the slight or entire absence of transmission, the missing factors may be supplied and cases occur. Although there is so far no canine filariasis reported in Minnesota, there is always danger of propagation if some infected dogs are imported. Twelve local species of mosquitoes, in some of which the filarial larvae were traced to the infective stage, were demonstrably susceptible. In five other local species, the numbers of mosquitoes tested were too few to make a complete examination, but seemed likewise to be susceptible. It is, therefore, possible that when all conditions are favorable, transmission will then be common.

Bibliography

Bahr, P. H. 1912 Filariasis and elephantiasis in Fiji. J. Trop. Med. London. Suppl. I.

Brug, S. L. 1916 Die schwarzen Sporen (black spores) bie der Malaria-infektion im Mückenkörper. Arch. Protistenk. **36**: 188–197.

Fülleborn, F. 1929 Filariosen des Menschen. Handb. path. Mikroorganismen von Kolle, Kraus und Uhlenhuth. **6**: 1043–1224.

GRASSI, B., AND NOÈ, G. 1900 The propagation of the filariae of the blood exclusively by means of the puncture of peculiar mosquitoes. Brit. Med. J. **2**: 1306–1307.

HU, S. M. K. 1931 Studies on host-parasite relationships of *Dirofilaria immitis* Leidy and its culicine intermediate hosts. Am. J. Hyg. **14**: 614–629.

HYLKEMA, B. 1920 De Ontwikkeling van de Parasiet der Quartana in de *Mysomyia ludlowi* en haar Overbrenging op den Mensch. Meded. Burg. Geneesk. Dienst Ned.-Indie, Batavia. No. 6, pp. 50–99.

JAMES, S. P. 1926 Epidemiological results of a laboratory study of malaria in England. Tr. Roy. Soc. Trop. Med. and Hyg. **20**: 143–165.

KNOWLES, R., AND BASU, B. C. 1933 The nature of the so-called "Black Spore" of Ross in malaria-transmitting mosquitoes. Indian J. Med. Res. **20**: 757–776.

MANSON-BAHR, P. H. 1921 Manson's Tropical Diseases. 7th edition. London.

MAYNE, B. 1929 The nature of the "Black Spores" associated with the malaria parasite in the mosquito and their relationship to the tracheal system. Indian J. Med. Res. **17**: 109–134.

NOÈ, G. 1900 Sul cicle evolutive della *Filaria bancrofti* (Cobbold) e della *Filaria immitis* (Leidy). Ric. Lab. Anat. norm. R. Univ. Romma ed in al. Lab. Biologici, fasc. 3 and 4.

SAMBON, L. W. 1902 Remarks on the life history of *Filaria bancrofti* and *Filaria immitis*. Lancet **163**: 422.

WELCH, E. 1921 Sur les "Black Spores" et autres corps chitinises dans les Anopheles. Compt. Rend. 4th Cong. Far East. Assn. Trop. Med., p. 448.

THE DOUBTFUL RELATIONSHIP OF SPOROZOA TO THE ULCERS OF *FUNDULUS HETEROCLITUS* (LINN.)*

FRANKLYN F. BOND

INTRODUCTION

In reviewing the literature on myxosporidiosis in *Fundulus heteroclitus* one immediately encounters the experiments conducted by Hahn at Woods Hole, Mass. His three papers (Hahn, 1913, 1917a, b) dealt with an attempt to determine the causative agent of severe sores occurring on these fish and to describe the stages of the infecting organism. He concluded that the form inhabiting the gill was the same as that occurring in the flesh.

Hahn's experiments in the main consisted of the introduction of infected tissues under the epidermis and into the flesh. Sores resulting from these infections caused the rapid death of the fish. These sores he compared with those appearing on the fish after they were brought into the laboratory. Although the spores figured by Hahn are myxosporidian spores, one cannot entirely agree with many of the other figures of developmental stages that he obtained from the sores of *F. heteroclitus*. Due to a lack of material infected with any form of *Myxobolus* similar to *Myxobolus funduli* Kudo this phase of the work could not be repeated. However, since Kudo considered the form in the gills to be a different genus and species, the presence of the same form, *Myxosoma funduli* Kudo, permitted a series of experiments including several similar to those carried out by Hahn. The presence of the same type sore allowed observations as to the nature of the ulcers and the agents causing them.

These studies are divided into two parts: (1) studies on the sores occurring on *F. heteroclitus* and attempts to determine the causative agent; (2) tests to determine the infectibility of these parasites by the dermal route, including repetition of Hahn's experiments.

MATERIAL AND METHODS

The *F. heteroclitus* used in this study were obtained from the Chesapeake Bay at Baltimore, Md., during the period October 1935 to March 1937. Parasites were abundant in the greater percentage of the fish, with *Myxosoma funduli* Kudo, *M. subtecalis* Bond, *Myxobolus bilineatum* Bond, and *Myxidium folium* Bond present in most (Bond, in press). The fish were kept in five-gallon aquaria during all experiments

Received for publication June 1, 1937.

*From the Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University. The writer is indebted to Dr. Robert Hegner for aid and advice in the presentation of this material.

and fed daily. Tissues were studied by smear, using Giemsa, Gram stain and methylene blue, and by section using iron hematoxylin or Mallory's triple connective tissue stain.

STUDIES ON THE OPEN SORES OF *F. heteroclitus*

Sores which often appear on *Fundulus* kept in aquaria have the appearance described by Hahn (1913). They have no definite localization and appear to result from rough handling or other injury to the protective mucous coat. In general the progress of the sores was found to follow the description given by Hahn and is somewhat similar to the common infections of game and aquarium fish in fresh water. The disease is much more pathogenic than that found in fresh-water fish and is definitely associated with the presence of a short, thick diplobacillus. Hahn correctly states that these infections may be cured with proper care and feeding. Since this may be also done by increasing the salinity of the water and by methylene blue treatment under isolation, the sores act much more like bacterial than protozoan infections. The nature of these infections in the *Fundulus* of Chesapeake Bay will be more clearly shown in the following studies.

The progress of the disease, when not disturbed by chemical treatment, may be divided into three stages. The first or superficial stage is characterized by the presence of a fine white fungus over the site, raised scales and a slight swelling. Smears at this stage, after staining with Giemsa, methylene blue or Gram stain, show long slender bacilli in great numbers, together with a fungus growth. The bacteria are Gram positive. Sections of the tissue after staining with Harris hematoxylin show pathological changes concentrated in the dermis and subcutaneous tissues. Bacteria of the long form are abundant throughout these areas, infiltration is heavy, and the muscle directly beneath the sore tends to show slight or no striation.

Intermediate stages in the development of the sores are characterized by the increased density of the fungus, loss of scales and the presence of a slight amount of blood oozing from the sore. The amount of blood lost during this stage of the infection varies. If the fins are involved there is usually a sufficient amount to give bloody streaks on the fins. Stained smears show both the long and the short Gram positive bacilli, the former exceeding the latter in abundance. Sections show increased muscle degeneration, bacteria in the muscular tissue, and numerous spaces filled with blood cells and pyknotic tissue nuclei. All cells not definitely of tissue or leucocytic origin were studied carefully but none could be associated with a sporozoan infection. Many degenerate cells suspected of being foreign in nature were much larger than any found present in

the early stages of *Myxosoma funduli* and larger than any of the somatic nuclei of the other myxosporidian species present in these fish.

The lesions during the later stages of the infection are open, deep wounds that penetrate into the muscle. When located on the head these reach to the skeletal structures and lay bare large areas of the bony frame, as was observed by Hahn. In such wounds, when smears are made from the deeper tissues, one can usually demonstrate a nearly pure culture of the short, thick, Gram positive bacilli. Smears from the edges of the wound may show some of the long bacilli. After the short form has obtained a supremacy over the longer form (as determined by abundance in smear) the progress of the infection is rather through the muscular tissue than a continued invasion of the muscle from the surface. Sections show extreme pathological changes with abundance of blood cells including phagocytes. Muscle fibers from near the wound show loss of striation suggesting early hyalinization. No cells of myxosporidian nature could be found in these lesions.

STUDIES ON THE EXPERIMENTS OF HAHN

In Hahn's experiments the infection of *F. heteroclitus* by insertion of infected tissue under the skin resulted in intensive sores, supposedly caused by the introduced parasites. Some (Hahn, 1917: 100) resulted in acute cases of infection, the fish dying in four days. He found: "The muscle fibers of the fish were abundantly infected with numerous small multiplicative trophoblasts, many large trophoblasts and also large masses of multinucleate sporoplasts." This infection was initiated by the insertion of entire infected gill filaments under the integument of a 6½ inch *F. heteroclitus*. Such rapidity of the development of the infection is remarkable when ascribed to myxosporidia, the information in the literature being considered. Thelohan (1894) earlier attempted to infect fish with a histozoic form by the injection of the parasites under the skin, with negative results, and he concluded that the method of infection was not by skin penetration.

The following experiments are attempted duplications of those performed by Hahn (1913, 1917) when he sought to determine whether *Myxosoma funduli* Kudo found in the gill filament was the same as a species of *Myxobolus* (*M. funduli*) that he described from the muscles of *F. heteroclitus*. These experiments were the basis of his statement (Hahn 1917) that there was only one species of myxosporidia to be found in *F. heteroclitus*. However, Hahn did note differences in morphology between the forms occurring in the gills and in the flesh. Kudo (1918) placed the gill form in another genus and described it as a new species, *Myxosoma funduli* Kudo.

Experiment 1. Infection by abrasion or cut.

Procedure: Eight freshly obtained *F. heteroclitus* were isolated and observed for a week. The fish were fed liver on alternate days and at the end of that period showed no indications of the open sores described in the preceding pages. On the seventh day of isolation, using a sterile scapel, an incision was made in the side of each fish, anterior to the dorsal fin and above the lateral line. The fish were then returned to their aquarium for observation another week.

Results: Three hours after the incisions most of the fish showed an excess of mucus secretion about the edges of the incision. After 8 hours there was a noticeable growth of white fungus present and an evident swelling. The next morning five of the fish were considerably swollen about the incision and slightly infected with the fungus. The other three, with only a slight swelling, were evidently recovering. All ate readily of liver when this was offered and showed no impairment in activity. Two days after the operation six of the fish were apparently approaching normal. They eventually recovered. The remaining two were killed while still heavily infected. The lesions were studied with the aid of smears and sections stained by Giemsa and hematoxylin. No evidence of a myxosporidian infection was found as a result of exposure of the incision to contamination from the water. Evidences of bacterial infection were abundant. All of the changes observed were similar to those already described.

Experiment 2. Attempts to infect fish previously exposed to infection by incision, by introducing infected gill tissue into a cut.

Procedure: Similar incisions but on the opposite side of the body were made in the six fish remaining from the preceding experiment. A piece of infected gill tissue bearing many cysts of *Myxosoma funduli* Kudo, was introduced into the incisions of three of the fish. These were paired off two fish per five-gallon tank, one bearing the simple cut, the other the cut into which the tissue had been introduced.

Results: The progress of the infections at first was similar to that in the preceding experiment. About 24 hours after the incisions were made, the fish with simple cuts began to recover while those into which infected tissue had been introduced, became steadily worse. All three of the latter died on the 3rd and 4th days. Infected areas had become enlarged, covered with fungus and of an unhealthy, whitish appearance. The tissues were very soft and mushy when touched. Sections and smears of the infected areas were stained with Giemsa, iron hematoxylin, and Mallory's triple connective tissue stain. No preparation showed active myxosporidian infection. Two smears contained a few spores but these had extruded no filaments and the sporoplasm and nuclei were still evident. Bacterial infection was present.

Experiment 3. Attempted infection of freshly obtained fish by insertion of infected gill tissue into a cut.

In this case experiment 2 was repeated with fish not previously weakened by incisions. The results were the same in the three fish into which pieces of tissue carrying cysts of *Myxosoma funduli* were inserted. The other three fish survived.

Experiment 4. Attempted infection of freshly obtained fish by the introduction of infected fin membrane into an incision.

Procedure: Incisions were made in six fish with a sterile scapel. Pieces of fin membrane containing cysts of *Myxosoma subtecalis* were inserted into the cuts on three of the fish. The experiment was continued by pairing the fish as in Experiment 2.

Results: Infections proceeded as in Experiment 2. All three fish into which the tissue had been introduced died with extended infections by the 4th day. One control died but was found negative for any myxosporidian infection.

The following types of experiments were not performed by Hahn, and tend to demonstrate more clearly the role of bacterial action in the preceding experiments.

Experiment 5. Study of the reaction following the introduction of non-infected gill tissue into incisions.

Procedure: Four *F. heteroclitus* were incised as described in the above experiments. Pieces of gill tissue from a small aquarium fish, *Xiphophorus helleri,* were placed in incisions in two of the fish.

Results: These two fish suffered the usual infection and died on the 3rd and 4th days. Controls were back to normal on the 5th day. The infected tissues were examined by smear and section. Evidences of bacterial infection only could be found. Infection was much advanced and nearly half the musculature on one side of the fish was destroyed.

Experiment 6. Attempted infection by subdermal injections of spore emulsions.

Procedure: Emulsions of spores from cysts of *Myxosoma funduli, Myxosoma subtecalis,* and *Myxobolus bilineatum* were made in saline. Using a small hypodermic needle, two fish were injected with *M. funduli,* one with *M. subtecalis* and one with *M. subtecalis* and *Myxobolus bilineatum.* Four fish were injected with saline and kept as controls. Previous to injection smears of the inoculum showed a large number of spores that were in the binucleate stage, although many of the *M. funduli* spores were mononucleate.

Results: During five days the fish were under observation there was no evidence externally of infection. On the 6th day the fish were killed, smears made and sections taken through the injected area. With Mallory's stain spores were found lodged subdermally. These were all

normal in structure and contained mono- or binucleate sporoplasms. A few spores were extruded but these still contained the sporoplasm nuclei. There was a slight amount of host cell infiltration to indicate the site of injection. No species of parasites injected gave indication of activity. No reaction was noted in the controls.

Experiment 7. Attempted infection by injection of spores into the peritoneal cavity.

Procedure: A large amount of emulsion of *Myxosoma subtecalis* from the brain was injected into the body cavity of three fish. One fish was examined in three hours, the second in 24 hours and the third at the end of 36 hours. Smears were made of the peritoneal fluid and stained with Giemsa.

Results: Spores were recovered in all cases. Extruded spores were common in smears made at the end of 24 and 36 hours. However, in all but a very few cases spores contained the sporoplasm nuclei. Several spores were seen where the valves had opened but the majority gave no evidence of activity.

DISCUSSION

In experimental studies on the myxosporidia of *Fundulus heteroclitus* (Linn.) the presence of superficial sores on these fish is explained and the sores themselves described in detail. The progress of the disease may be divided into three stages, each with certain features relating to the presence of causative agents. Experiments definitely showed that lesions described by Hahn may be readily produced by other means than by the introduction of parasites. Further it was shown that injections of the spores themselves did not produce any visible infection, and no evidence was found pointing to parasitic activity in cases where *Myxosoma funduli* Kudo, *M. subtecalis* Bond, and *Myxobolus bilineatum* Bond were injected subdermally or into the peritoneal cavity. Study of the infections caused by the insertions of tissue (infected or non-infected) into incisions in the skin showed pathological pictures very similar to those in sores where only bacterial infections were found.

CONCLUSIONS

1. *Fundulus heteroclitus,* taken from Chesapeake Bay, may develop sores in which two bacterial forms, one a long and slender bacillus and the other a short, thick diplobacillus, both Gram positive, were consistently found.

2. In no case were myxosporidian spores or developmental stages present that could be suspected of causing the sores.

3. The sporozoan of the gill, *Myxosoma funduli* Kudo, was not infective to the flesh of *Fundulus* by direct introduction of the parasites.

Myxosoma subtecalis Bond and *Myxobolus bilineatum* Bond were also non-infective by similar introduction of the parasites.

4. There was no obvious connection between myxosporidian infection and the presence of the open sores on the *Fundulus* from Chesapeake Bay.

Bibliography

Bond, F. F. 1937 Cnidosporidia from *Fundulus heteroclitus* (Linn.). Tr. Amer. Micr. Soc. (in press).

Hahn, C. W. 1913 Sporozoan parasites of certain fishes in the vicinity of Woods Hole, Mass. Bull. U. S. Fish. Comm. **33**: 191–214.

———1917a Further observations on *Myxobolus musculi,* from *Fundulus.* J. Parasitol. **3**: 91–104.

———1917b On the sporozoan parasites of the fishes of Woods Hole and vicinity. II. Additional observations upon the *Myxobolus musculi* of *Fundulus* and a nearly related species, *Myxobolus pleuronectidae* of *Pleuronectes americanus.* J. Parasitol. **3**: 150–162.

Kudo, R. R. 1918 Contributions to the study of parasitic protozoa. IV. Notes on some of the Myxosporidia from certain fish in the vicinity of Woods Hole. J. Parasitol. **5**: 11–16.

Thelohan, P. 1894 Recherches sur les Myxosporidies. Bull. Sc. France et Belg. **26**: 100–394.

A NEW SPECIES OF TREMATODE, *DIASIA PODILYMBAE* (OPISTHORCHIIDAE), FROM THE PIED-BILLED GREBE, *PODILYMBUS PODICEPS* (LINN.)*

O. Wilford Olsen

From the mesenteries of a pied-billed grebe, a number of medium-sized trematodes was collected by Mr. J. F. Bell of the Bacteriology Department of the University of Minnesota who sent them to the writer for identification. They were found to constitute an undescribed species of the genus *Diasia* Travassos, 1922. Ova discharged into the retaining mesenteries had been ingested by the flukes thus densely packing their digestive tracts with them.

The genus *Diasia* as erected by Travassos was monospecific with *D. diasi* Travassos, 1922, as the type. Subsequent inclusion of additional species requires some slight emendation of the generic concept which is herewith given in its revised form.

Diasia Travassos, 1922 diagnosis emend.

Generic diagnosis: Pachytreminae. Suckers rudimentary, pharynx follows the oral sucker; genital pore preequatorial; cirrus pouch atrophied or absent; testes tandem or obliquely placed, lobed or smooth; ovary lobed; in the pancreas, intestine, or mesenteries of birds.

Type species: Diasia diasi Travassos, 1922.

Diasia podilymbae, n. sp.

(Figs. 1–7)

Specific diagnosis: Diasia. Body elongated, slender, somewhat thickened dorsoventrally, gradually narrowed anteriorly from region of acetabulum and terminating in a rather narrow point without a muscular development forming a thickened area; sides parallel caudad from acetabulum, posterior end distinctly truncated, ventral surface of body thrown into many strong, transverse rugae extending across body. Lengths of sexually mature specimens 3.6–10 mm; widths at regions of acetabulum and ovary 0.50–0.82 mm and 0.61–1.04 mm respectively. Cuticle without spines. Oral sucker terminal, rudimentary, width 0.070–0.122 mm, length 0.019–0.031 mm; acetabulum rudimentary, near posterior margin of first quarter of body length, diameter 0.020–0.037 mm. Prepharynx very short, pharynx conspicuous, pyriform, length 0.086–0.133 mm, width 0.082–0.109 mm; esophagus relatively short, rarely so long as one-third of the distance from the anterior end of body to the acetabulum, diameter great; intestinal caeca wide, extend to posterior extremity of body, usually filled throughout with ova. Genital pore minute, median, slightly anterior to rudimentary acetabulum for which it might readily be mistaken in whole mounts, particularly in uncleared specimens. Cirrus pouch absent, seminal vesicle large, dorsoventral in position, divided by constriction into a large thin-walled and a small thick-walled chamber which leads directly into the prostate portion, the latter surrounded by a few darkly staining glandular cells; cirrus without spines; testes tandem, caudad

Received for publication, August 23, 1937.

* Paper No. 1534 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station, St. Paul.

from ovary, located in posterior fifth of body and usually near posterior end, variously shaped and with margins smooth or uneven; size of anterior testis 0.104–0.261 mm wide by 0.131–0.313 mm long, posterior 0.156–0.348 mm wide by 0.122–0.383 mm long; vasa efferentia arise from anterior margin of testes; that from the anterior one passes to the right of the ovary and that from the posterior to the left, uniting dorsal to the uterus a short distance cephalad from ovary; vas deferens passes anteriorly along dorsal side of uterus to seminal vesicle. Ovary located near juncture of fourth and last fifths of body length, usually to right of median line but may be to left, lobulated, about size of seminal receptacle; oviduct short, union of oviduct, common yolk duct, and duct from seminal receptable occurs at one point; Mehlis' gland consists of loosely connected and rather widely separated cells; uterus extends cephalad from ovary, coils packed with ova and completely fill intercaecal space, frequently overlap caeca somewhat and terminate in a poorly developed metraterm; Laurer's canal not seen in whole mounts or sections; seminal receptacle large and conspicuous, dorsal and lateral to ovary, width 0.191–0.400 mm, which is somewhat greater than length. Vitellaria in well-developed chains lying laterally between body margin and intestinal caeca, slightly overlapping latter, beginning about 0.5 mm caudad from acetabulum, and extending to posterior extremity of body; transverse yolk ducts extend mesad near level of ovary, yolk reservoir absent. Excretory bladder sac-like, extends cephalad to level of ovary on ventral side of body; excretory pore subterminal, ventral. Ova 21–27 μ long by 10–12 μ wide.

Host: Podilymbus podiceps (Linn.).

Habitat: Mesenteries.

Locality: Minnesota, U. S. A.

Type specimens: U. S. Nat. Mus. Helm. Coll. No. 9057, paratype No. 9058; other paratypes in collection of Univ. Minn. Helm. Coll. and of author.

Diasia podilymbae differs from *D. diasi* Travassos, 1922, in being smaller in body size; in having larger suckers, both of which are visible in whole mounts; and in having the testes with the margins at most only slightly uneven instead of profoundly lobed. It differs from *D. fodiens* (Linton, 1928) in the absence of a distinctly muscular anterior extremity of subtriangular shape; in that the caeca terminate in the lateral regions of the body rather than being juxtaposed distally as in *D. fodiens;* and in that the caudal extremity of *D. podilymbae* is markedly truncated instead of rounded.

DISCUSSION

Travassos (1922) erected the genus *Diasia* to receive a new species, *D. diasi,* of OPISTHORCHIIDAE found in the pancreas of the water-turkey (*Plotus anhinga*) and assigned the genus to the subfamily PACHYTREMINAE. Under the name *Haematotrephus fodiens,* Linton (1928) described a species of trematode infecting the digestive tract and pancreas of the loon (*Gavia immer*). In his description, he pointed out the fact that the extremities of the intestinal caeca, while in contact, are not actually continuous as in members of the CYCLOCOELIDAE. Ejsmont (1931) transferred *H. fodiens* Linton to the genus *Diasia.*

BIBLIOGRAPHY

EJSMONT, M. L. 1931 Sur l'identité de *Proshystera rossittensis* Korkhaus et *Tanaisia fedtschenkoi* Skrjabin avec quelques remarques sur les Trématodes

aux caecums réunis. Acad. Polonaise Sc., Lett. C. R. Mensuels Cl. Sc. Math. et Nat. Cracovie No. 6, p. 7.

Linton, Edwin. 1928 Notes on trematode parasites of birds. Proc. U. S. Nat. Mus. **73**: 1–36.

Travassos, L. 1922 Informações sobre a fauna helminthologica de Matto Grosso. Folha Medica **3**: 187–190.

Explanation of Plate, p. 218

Drawings made with the aid of a camera lucida.

Fig. 1. Sexually mature specimen of *Diasia podilymbae*. Dorsal view.
Fig. 2. Sagittal section through posterior region of body. Dextral view.
Fig. 3. Sagittal section of body through region of acetabulum. Sinistral view.
Fig. 4. Mature ovum.
Fig. 5. Transverse section of body at level of ovary and seminal receptacle.
Fig. 6. Anterior extremity.
Fig. 7. Organs of female reproductive system. Cephalic view.

Abbreviations

AC	acetabulum	P	pharynx
CYD	common yolk duct	PG	prostate gland
EB	excretory bladder	SR	seminal receptacle
GP	genital pore	SV	seminal vesicle
IN	intestine	T	testis
MG	Mehlis' gland	UT	uterus
O	ovum	V	vitellaria
OE	esophagus	VD	vas deferens
OV	ovary	VE	vasa efferentia
OVD	oviduct	YCD	yolk collecting duct

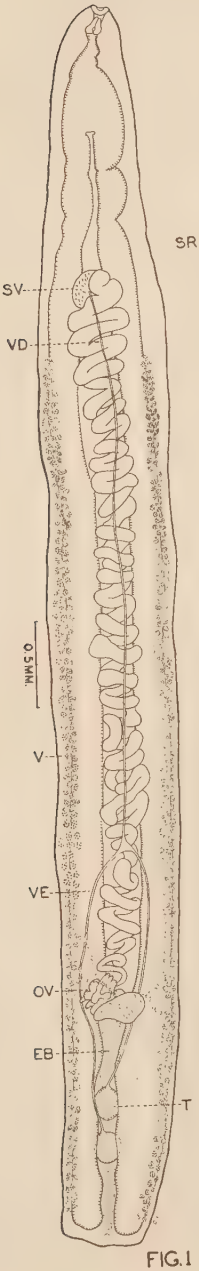


FIG. 1

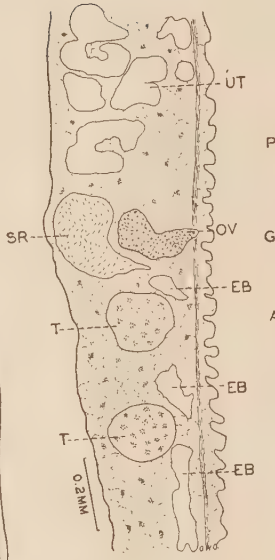


FIG. 2

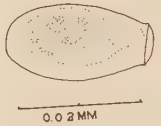


FIG. 4

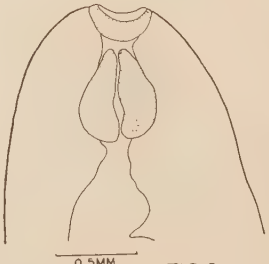


FIG. 6

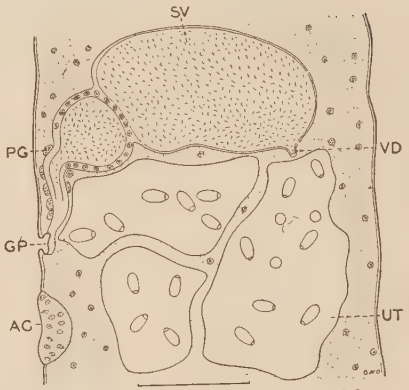


FIG. 3

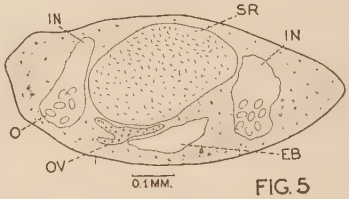


FIG. 5

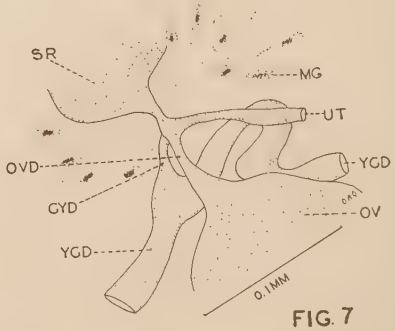


FIG. 7

Diasia podilymbae

THE MORPHOLOGY AND LIFE CYCLE OF *EURYHELMIS MONORCHIS* N. SP. (TREMATODA) FROM THE MINK*

DONALD J. AMEEL

Over a period of years, a small heterophyid trematode was collected from minks examined in the helminthology laboratory. The material received little attention until identical specimens were recovered from white rats that had been fed metacercariae developed experimentally in green frogs, *Rana clamitans* Latreille. Superficially the flukes resemble *Euryhelmis squamula* (Rud., 1819), a common parasite of the polecat, *Putorius putorius,* in certain parts of Europe. However, a microscopic examination of stained specimens revealed a complete degeneration of the male genital system in a majority of the flukes. A study of a large number of specimens indicates that there is but one testis which is transitory, it being present for an exceedingly brief period during the life of the adult fluke. This peculiar morphological condition suggested the specific name *monorchis.* The inclusion of this species in the genus *Euryhelmis,* where it undoubtedly belongs, necessitates a modification of the original generic diagnosis. Grateful acknowledgement is due Dr. George R. La Rue who made this study possible.

Euryhelmis Rudolphi, 1819, diagnosis emend.

Generic diagnosis: Body small, leaflike, much broader than long. Excretory vesicle Y- or T-shaped. Testes one and transitory, or two, spherical or lobate, in posterior half of body. Cirrus-pouch absent. Seminal vesicle present or absent, when present, large and persisting throughout life of adult. Uterus relatively short, consisting of three loops situated principally on left side of body between ventral sucker and excretory vesicle. Vitelline follicles numerous, mainly lateral, extending from posterior region of body to intestinal bifurcation. Genital atrium immediately in front of ventral sucker. Intestinal crura extend to posterior extremity of body. Eggs operculate, with or without slight polar thickening. Adults in intestine of mustelids.

Type species: Euryhelmis squamula (Rudolphi, 1819).

Euryhelmis monorchis n. sp.

(Fig. 5)

Specific diagnosis: Body thin, leaflike, transparent, usually much broader than long, spinose. Length, 0.44 (0.39–0.46) mm; greatest width, 0.61 (0.52–0.69) mm. Oral sucker 0.041 (0.032–0.048) mm in anterior-posterior, 0.059 (0.048–0.672) in transverse diameter. Ventral sucker 0.060 (0.040–0.064) mm in anterior-posterior, 0.069 (0.062–0.076) mm in transverse diameter. Gonotyl ("genital sucker") about 0.032 by 0.048 mm. Pharynx large, spherical, impinging on oral sucker, 0.033 (0.032–0.040) mm by 0.035 (0.032–0.043) mm. Esophagus slender, bifurcating close

Received for publication, August 26, 1937.

* Contribution of the Department of Zoology and the Biological Station of the University of Michigan.

to ventral sucker. Intestinal crura extending diagonally to sides, then to posterior region of body. Testis one, small, spherical, on right side near posterior end of body. Seminal vesicle absent. Ovary dextral, elliptical or lobate. Seminal receptacle large, between ovary and excretory bladder. Uterus usually consisting of three loops, confined to region limited by ventral sucker, excretory bladder, ovary and left limb of intestine. Laurer's canal present. Mehlis' gland well developed. Vitelline follicles numerous, largely lateral, extending from stem of excretory bladder to bifurcation of intestine. Eggs operculate, undeveloped when laid, 29 (25–30) by 14 (12–14) μ. Excretory bladder Y-shaped, large and conspicuous.

Cercaria (Fig. 4) : Lophocercous, spinose. Measurements of 10 cercariae killed in hot 10 per cent formalin: total length, 0.36 (0.33–0.39) mm; body length, 0.11 (0.10–0.12) mm; body width, 0.04 mm; tail length, 0.25 (0.23–0.27) mm. Oral sucker, 0.022 (0.020–0.024) mm in anterior-posterior, 0.022 (0.020–0.022) mm in transverse diameter. A small cluster of cells midway between oral sucker and penetration glands may be interpreted as the undeveloped pharynx; no other evidence of a digestive tract. Penetration glands about 12 in number, in third fourth of body. Ducts proceeding forward in a central mass, bifurcating shortly before reaching oral sucker, each branch bifurcating again at posterior edge of sucker. Excretory bladder large, broadly V-shaped. Genital primordium between excretory bladder and penetration glands. Ventral sucker small, poorly developed, ventral to genital primordium.

Redia (Fig. 1) : Simple, without appendages, rugose to smooth, active. Birth pore present. Length, 0.67 (0.53–0.85) mm; width, 0.13 (0.12–0.15) mm. Pharynx, 0.032 (0.032–0.033) mm by 0.032 (0.032–0.033) mm. Gut small, clear, 0.020 (0.014–0.028) mm by 0.020 (0.016–0.024) mm.

Hosts: Primary, *Mustela vision* Schreber and, experimentally, white rat and domestic cat; first intermediate, operculate snail, *Pomatiopsis lapidaria* Say; second intermediate, *Rana clamitans* Latreille and, experimentally, *R. pipiens* Shreber and *R. palustris* Le Conte.

Location: Small intestine of primary host.

Distribution: United States—Wisconsin, Ohio (Napoleon) and Michigan (Jackson, Ann Arbor, Flushing and Whitehall).

Type specimen: U. S. Nat. Mus. Helm. Coll. Cotype, Dept. Zool., University of Michigan.

Though *Euryhelmis squamula* was poorly described it has long been recognized as a member of the family HETEROPHYIDAE. Witenberg (1929) regarded its description as being so inadequate that he excluded this genus from his monograph of the family. However, a complete description of *E. squamula* has been published recently by Baer (1931), which definitely fixes its taxonomic position.

Witenberg placed the genera of the family HETEROPHYIDAE in five subfamilies. According to this classification *Euryhelmis* belongs to the subfamily HETEROPHYINAE. The presence of but one testis in *E. monorchis* runs counter to the original conception of the subfamily. Nevertheless, the nature and relative position of this testis are such as to indicate that *E. monorchis* is probably an aberrant form.

GENERAL OBSERVATIONS

The cercaria of *E. monorchis* is one of several studied from the snail *P. lapidaria*. It was present in 5.1% of the snails collected near

Ann Arbor, Michigan. Though an active swimmer, the periods of activity are alternated with periods of rest when the cercaria sinks to the bottom in a characteristic flexed position (Fig. 2). It lacks several features typical of heterophyid cercariae, namely, eyespots and specialized spines at the oral aperture. The presence of these spines has been recognized by some as a means of distinguishing heterophyid cercariae from cercariae of the OPISTHORCHIIDAE which they greatly resemble.

The tail is several times longer than the body. The fin fold, which is easily observed with weak illumination, extends from near the base of the tail on the dorsal surface to the tip and is present on the posterior third of the ventral aspect. The tip of the tail is very contractile and is in continuous motion in an active cercaria.

The internal anatomy was studied with the aid of aqueous solutions of neutral red and light green. Numerous cystogenous glands obscured some structures. Neither the penetration glands nor their ducts were well defined. The genital primordium when stained with neutral red appeared as a large pink mass immediately posterior to the penetration glands. The ventral sucker could be discerned with difficulty in any but well stained specimens. However, it was noted as a conspicuous protuberance in well developed cercariae within the rediae. The excretory vesicle is large and conspicuous, in part due to the presence of refractile granules in its cells. Excretory tubules could not be traced. There are nine small flame cells on each side, with an apparent pattern of 2[2+2+3+2] (Fig. 3).

The cercariae readily penetrate frogs and tadpoles exposed to them. The skin at the points of entry soon becomes rather clear and raised like a blister. Later the presence of the cysts can be detected with the naked eye because of the swollen areas. They were present over the entire body of experimentally infected tadpoles but were confined largely to the limbs and lateral and ventral areas of the frogs. No naturally infected tadpoles were found, but 66% of the green frogs, *Rana clamitans,* collected where the cercariae occurred yielded metacercariae. These were located on the legs and lower portions of the flanks. Though leopard and pickerel frogs, *R. pipiens* and *R. palustris,* from the same area were negative, large numbers of metacercariae were recovered from these species infected experimentally.

The metacercariae locate in the skin and subcutaneous connective tissue. The cyst is composed of a thin inner membrane secreted by the cercaria and a thick pigmented layer of host tissue. Five, eighteen and forty-five day metacercariae within the inner membrane measured 0.11, 0.18 and 0.36 (0.32–0.45) mm in diameter, respectively. These last cysts are of maximum size and the metacercariae were infective. Fully developed metacercariae removed from the cysts, though of characteristic

form when alive, assumed a form longer than broad when killed with hot Bouin's or sublimate fixative. Ten of these stained and mounted on slides averaged 0.45 (0.40–0.52) mm by 0.34 (0.30–0.38) mm. A comparison of these measurements with those of full grown worms revealed that the adults were less than twice the size of the metacercariae. A testis was observed in but two of 23 mounted specimens.

It has long been known that *E. squamula* encysts in various species of frogs. The metacercariae were reported from the frog, *R. temporaria,* as early as 1867 by Zeller who found them in two thirds of the frogs collected near Tübingen, Germany. Joyeux, Baer and Carrère (1934) found them in all tadpoles and young adults of *R. esculenta* collected near Marseille, France. Though specimens of *E. squamula* from minks collected in Maryland and Minnesota have been in the helminthological collections of the U. S. National Museum for some time, the first published report of this trematode from the United States is by McIntosh (1936). He recovered the metacercariae from *R. pipiens* collected near Alexandria, Virginia, fed them to cats and recovered the adults which he identified as *E. squamula.*

Mature worms of *E. monorchis* were recovered from naturally infected minks and experimentally infected rats and a cat. Specimens from the rat averaged somewhat smaller than those found in the mink. Rats examined 3½ days after infection yielded specimens containing as many as 52 eggs. At that time all the worms were in the duodenum. In several rats examined 16 days after infection, a few worms were recovered from the duodenum but more were found in the jejunum. Because of its small size and its location among the villi of the intestine, there is no doubt that, except when it occurs in large numbers, this fluke is often overlooked in the examination of minks. Hundreds have been collected from a single mink. Those used in this study were collected by means of the shaking technique, viz., the intestine was slit open, placed in a jar containing cold sublimate acetic and the whole vigorously shaken. The worms were later recovered with the aid of a binocular microscope. The species description was based largely on this mink material.

The noteworthy characteristic of the adult worms is the transitory nature of the single testis and associated structures. The majority of the specimens lacked entirely a male genital system. Out of 255 worms which were stained and mounted or sectioned only 7 or 2.7% possessed a testis which was always on the same side of the body as the ovary. The anterior portion of the uterus was filled with sperm in some 3½ day old worms but none possessed a testis. The testis was present in four out of 48 four day old and two out of 105 sixteen day old worms. It was observed in but one of 102 specimens from the mink. The seminal receptacle was empty in all specimens.

It could be assumed that the male genital system fails to develop in the majority of the worms. However, a more logical explanation of this unusual condition is that the testis is present for a brief period in the life of the trematode, just long enough to produce and discharge a supply of sperm.

SUMMARY

1. The morphology and life-cycle of *Euryhelmis monorchis,* a small heterophyid from the mink, have been described.

2. Though this trematode has one testis which is transitory and no seminal vesicle, it is placed in the genus *Euryhelmis,* because with these exceptions, its characters are those of the genus.

3. The first intermediate host is the snail, *Pomatiopsis lapidaria,* while tadpoles and frogs serve as second intermediate hosts.

BIBLIOGRAPHY

BAER, J.-G. 1931 Helminthes rare ou peu connus du putois. Rev. Suisse Zool. **38**: 313–334.

JOYEUX, C., BAER, J.-G. AND CARRÈRE, P. 1934 Recherches sur le cycle évolutif d'*Euryhelmis squamula*. Compt. Rend. Acad. Sc. **199**: 1067–1068.

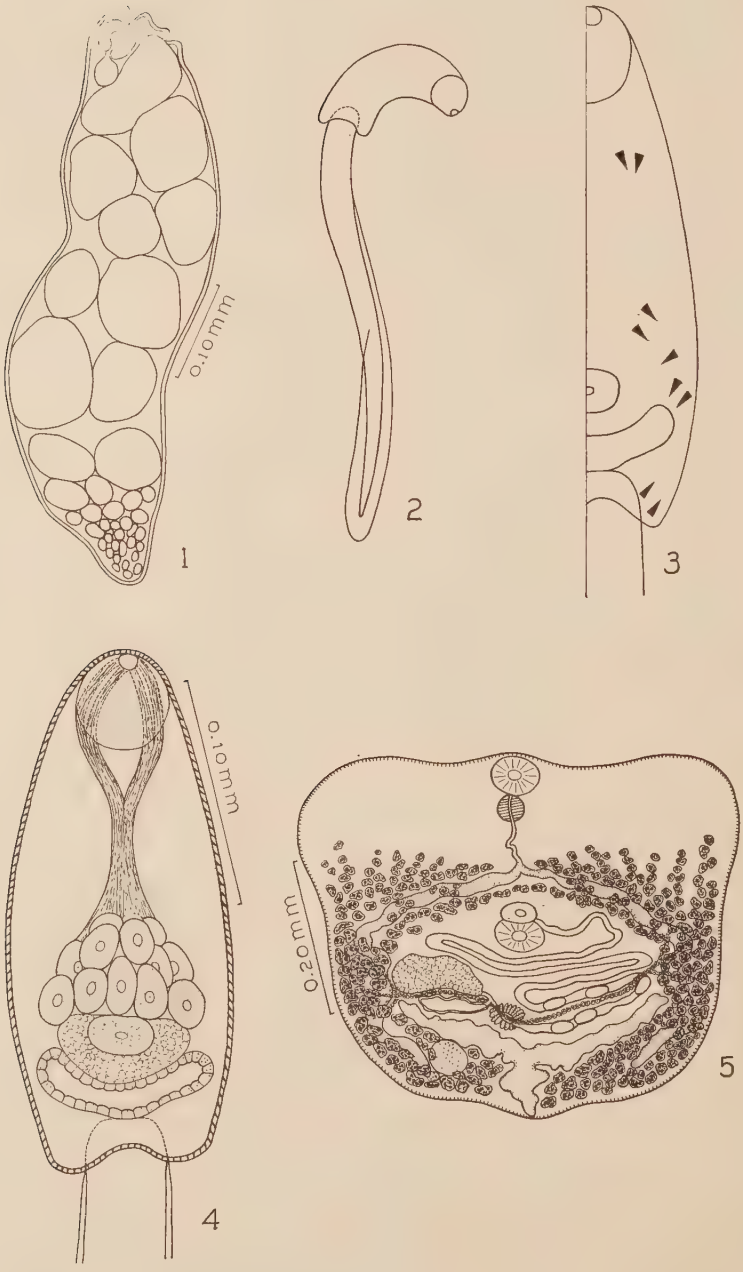
MCINTOSH, A. 1936 The occurrence of *Euryhelmis squamula* (Rud., 1819) in the United States. J. Parasitol. **22**: 536.

WITENBERG, G. 1929 Studies on the trematode family *Heterophyidae*. Ann. Trop. Med. and Parasitol. **23**: 131–239.

ZELLER, E. 1867 Ueber das enkystierte Vorkommen von *Distomum squamula* Rud. im braunen Grasfrosch. Z. Wissensch. Zool. **17**: 215–220.

EXPLANATION OF PLATE, P. 224

Fig. 1. Redia.
Fig. 2. Cercaria in resting position.
Fig. 3. Flame cell arrangement in cercaria.
Fig. 4. Cercaria.
Fig. 5. Young adult.



Euryhelmis monorchis

ON THE LOCALIZATION OF ADULT TRICHINAE IN THE INTESTINE*

HANS ROTH

That *Trichinella spiralis* in its adult stage prefers certain parts of the intestine is a question to which but little attention has been paid. Writers on this form mention only that trichinae inhabit the small intestine. According to McCoy (1931), who examined the small intestines of rats for trichinae, it is recognized that a few trichinae are sometimes found in the cecum; but, on account of the practical difficulty of examining these parts, which are full of foreign material, he did not include the cecum and large intestine in his investigations.

While investigating the immunity of guinea pigs to super-infection with *Trichinella spiralis* (to be published), I tried to find a reliable method of counting the number of adult trichinae in the intestine. In contrast to McCoy who counted the total number of worms by stripping the mucosa from the whole intestine, dissolving it in 0.04 per cent sodium hydroxide, and then testing 1 cc samples of the solution, I tried, so far as possible, to determine the number of the adult trichinae *in situ*. My procedure was, to cut the different sections of the intestine into small pieces, which were then measured. The mucosa on each piece, with the covering layer of feces, was then stripped off, put on a very large slide and pulled to pieces in 0.9 per cent saline solution by means of needles. The slide was carefully examined several times under a binocular microscope and the worms collected.

This method was, of course, very tedious and took a great deal of time, but the results attained by complete, or nearly complete, examination of only a few intestines showed a distinctly localized distribution of the trichinae. It was therefore possible, during the further investigations, to examine a series of samples from different sections of the intestine (as described below), as these samples give a clear idea of the relative extent of the infection. The length of the total samples taken from the small intestine of each animal was about 25 cm; from the cecum about 20 sq cm. No samples were taken, as a rule, from the large intestine. All the guinea pigs in my experiments were infected by the ingestion of larvae, the numbers of which were exactly counted under the dissecting microscope, after having been freed from their cysts by artificial digestion in a manner similar to that described by McCoy.

Altogether, more than fifty intestines have been examined, most of them by sampling, as described. Nearly all the animals revealed typical

Received for publication, September 25, 1937.

* From the Institute for General Pathology, University of Copenhagen. Director: Professor Oluf Thomsen, M.D.

distribution of adult worms in the intestine, a fact which, so far as the author is aware, has not been earlier noted.

It should first be emphasized, that the small intestine, at least in guinea pigs, is not the only habitat of adult trichinae. The cecum and the beginning of the large intestine are also crowded with live and fully developed worms, the majority of which have invaded the mucosa in the same way as occurs in the small intestine. While there are not many trichinae in the large intestine, the worms being only in the first section nearest the cecum, it has been shown that there are quantities of them in the cecum, where they are to be found spread over the whole surface. The heavier the dose of infection, the greater the relative number of trichinae inhabiting the cecum (and large intestine), as compared with the number of those that have settled in the small intestine. When the total intestine of guinea pig No. 38, which had been given a dose of 750 larvae, was examined 17 days after infection, it was found to contain 229 adult trichinae, i.e., 134 worms in the small intestine, 74 in the cecum and 21 in the large intestine. Other animals, which had been infected with doses of 1500 or 2000 larvae each and the intestines of which were examined either in total or by sampling, showed approximately the same number of trichinae in the small intestine as were in the cecum and the beginning of the large intestine. In the cases, however, in which massive doses of about 6000, 12,000 or 18,000 larvae had been given, the infection in the cecum seemed to be greater than that in the small intestine (see table 2).

Although the general statement might be made, at least as far as concerns guinea pigs, that there are as many trichinae in the cecum and the large intestine as in the small intestine, there are indications that adult trichinae which have settled in the cecum do not play so large a part in the propagation of young larvae throughout the host as those which have settled in the small intestine. Samples were also taken from the striated muscles of each animal whose intestine was examined. These were pressed between two thick glass slides and examined under the microscope. A comparison between the results obtained from examination of the intestine and those from examination of the muscles showed that the number and age of larvae that had immigrated into the muscles were in many cases dependent on the presence of adult worms in the small intestine rather than in the cecum. Differences in histological structure and vascularisation between the different sections of the intestine may perhaps make it more difficult for the young larva coming from the cecum to invade the circulation, though it cannot be thought that the cecum plays no part in the propagation of young trichinae.

It can easily be seen from the foregoing that the finding of a distinctly localized distribution of adult trichinae in the small intestine is of

theoretical and practical value. It could be shown that the trichinae in all the animals examined were, for the most part, crowded in the last section of the small intestine, the greatest concentration being particularly noticeable in the last few centimeters of the posterior end, where the small intestine discharges into the cecum. From this point of predilection forwards the number of trichinae decreases very rapidly. Table 1

TABLE 1.—*Examination of a series of samples from the intestines of two guinea pigs, each infected with 1,500 larvae*

	Small intestine examination starting at the posterior end			Cecum	
	Distance between samples in cm.	Length of samples in cm.	Number of trichinae	Size of samples in sq. cm.	Number of trichinae
Guinea pig No. 106, 9 days after infection		3	60	7.5	6
		2.5	29	4	2
		2	20	4.5	12
		2	9	4	14
	3	1	3		
	30	3.5	2		
	30	2.5	2		
	30	3	0		
	25	2.5	0		
		3	0		
	Total ...	25	125	20	34
Guinea pig No. 115, 20 days after infection		3.5	64	3.5	2
		4	44	3.5	8
		2.5	7	5.5	16
	30	3	1	4	4
	30	3	1	3.5	7
	30	3	0		
	13	3	0		
		3	0		
	Total ...	25	117	20	37

shows two typical examples of an experiment in which both guinea pigs were given the usual dose of 1500 larvae each and killed 9 and 21 days after infection. The intestines were examined by taking the usual series of samples. From the small intestine, the total length of which varied from 125 to 160 cm, altogether 25 cm of samples were taken: 10 cm from the posterior end, one or two samples about 3 cm in length from the anterior end, and a few from the remainder of the organ. The distance between the single samples was generally about 30 cm. From the cecum, the area of which varied from about 80 to 120 sq cm, 20 sq cm of samples were taken from different parts. As may be seen from table 1, the results from both animals were very similar. The average number of trichinae per centimeter intestine, at the posterior end of the small intes-

tine 20 and 18.3, respectively, falls very rapidly even in the next 10 cm to 4.5 and 2.8. Then, further forward, where there were distances of about 30 cm between the single samples, the trichinae became very rare, their average number per centimeter varying from 0.3 to 0.8. No worms were observed in the samples from the anterior part of the small intestine.

In the cases in which massive doses of larvae had been given the concentrations of adult worms at the posterior end of the small intestine and in the cecum were enormous. Trichinae were also found sparsely distributed all through the small intestine, as can be seen from table 2,

TABLE 2.—*Examination of a series of samples from the intestine of guinea pig No. 209, infected with 6,250 larvae and dying 6 days after infection*

Small intestine examination starting at the posterior end			Cecum	
Distance between samples in cm.	Length of samples in cm.	Number of trichinae	Size of samples in sq. cm.	Number of trichinae
	2	76	2.5	33
	2	29	4	79
	2	10	3	87
	4	15	3.5	74
30			4	87
	3	2	3	41
30				
	3	3		
30				
	3	1		
30				
	3	4		
3				
	3	2		
Total	25	142	20	401

which shows the results obtained from a guinea pig infected with 6250 larvae which died 6 days after infection. On the other hand, in cases where there was only slight total infection, nearly all the trichinae in the small intestine were accumulated at the posterior end. Table 3, shows the results obtained from a guinea pig infected with 300 larvae and killed 12 days after infection.

The fact that adult trichinae prefer a certain place in the posterior end of the small intestine of the guinea pig is of definite diagnostic value. The search for adult trichinae within the whole intestine, especially in cases of slight infection, was very tedious on account of the thinness of the worms. It will now be possible by examining only the last few centimeters of the small intestine to decide rather definitely whether any infection is present.

It might perhaps be imagined that the main concentration of adult trichinae in the last part of the small intestine and in the cecum was not of natural occurrence but was caused by the 24 hours' starvation which most of the animals underwent before death, or by the postmortem

TABLE 3.—*Examination of a series of samples from the intestine of guinea pig No. 159, infected with 300 larvae and killed 12 days after infection*

Small intestine examination starting at the posterior end			Cecum	
Distance between samples in cm.	Length of samples in cm.	Number of trichinae	Size of samples in sq. cm.	Number of trichinae
	2	4	3.5	0
	2	2	2.5	1
	3	0	3	1
	3	2	5.5	1
30			5.5	5
	3	0		
30				
	3.5	0		
30				
	3	0		
30				
	3.5	0		
15				
	2.5	1		
Total	25	9	20	8

changes which occurred when the intestines were not examined immediately after death but were preserved in the refrigerator. However, the fact that the majority of the trichinae were found deeply embedded in the mucosa alone indicates that their position was the same as it was in the live animal. Furthermore, some individual animals, which had not been starved, and from which the samples were taken immediately after they had been killed, showed the same results.

It must therefore be considered as demonstrated that by far the majority of the adult trichinae present in the intestines of guinea pigs are accumulated in the posterior part of the small intestine and in the cecum. In this connection, it is worth mentioning that one large and a few smaller mesenteric lymph nodes are situated near the junction of the small intestine and the cecum. This juxtaposition must be significant in the relation of the mesenteric lymph nodes to the migration of the young trichina larvae coming from the intestine.

The longevity of the adult trichinae in the intestine seems to vary greatly for different hosts. According to McCoy (1931, 1932), rats show a certain degree of natural resistance to trichinosis, the adult worms only living in the intestine for two weeks, and the production of young larvae, therefore, being relatively small. Large doses may break down this natural resistance, so that the adult trichinae can live in the intestine for 4 or 5 weeks. Matoff and Wapzarowa (1937) and Matoff (1937) found adult trichinae living in the intestines of mice for 16 days and in the intestines of dogs for only 10 days.

In accordance with McCoy's statement it was proved that rabbits react in almost the same way as rats, while guinea pigs show much less resistance to this infection, the adult trichinae living in the small intestine

for from 30 to 37 days and in the cecum for from 42 to 55 days, regardless of the size of the dose ingested. As males and embryo-producing females, mostly embedded in the mucosa, can be found during the whole period, it will be easily understood that the effectiveness of even small doses proved to be much higher as regards the final number of larvae in the muscles of guinea pigs, than was the case for rats, rabbits, mice, dogs and other animals. In one extraordinary case (guinea pig No. 129, infected with only 100 larvae) living males and embryo-producing females were found in the posterior end of the small intestine as long as 50 days after infection, and at the same time samples from the muscles showed young stages as well as encysted larvae. The immigration of young larvae into the muscles generally began from the 6th to the 8th day after infection and ended from the 30th to the 37th day, the conclusion corresponding very largely to the disappearance of the adult worms from the small intestine. These questions will be dealt with more fully in a later publication.

The length of the fully developed male, adult trichina is generally taken to be 1.4 to 1.6 mm and that of the females 2.2 to 3.6 mm. But as the trichinae live so long in the intestines of guinea pigs, their length, too, may become greater, the males reaching 2.0 to 2.2 mm and the females 4.0 to 4.8 mm, as in guinea pig No. 129, mentioned above, in the small intestine of which the trichinae had been living for 50 days.

On the other hand, when large doses of larvae were given, (as in the case of guinea pig 209, table 2), with enormous accumulations of adult trichinae especially at the very end of the small intestine, the growth-rate of the worms in this section was greatly restricted. Although sexually mature, many of the males measured only 0.6 to 0.8 mm and many of the females only 1.1 to 1.6 mm. The length of the worms in samples from the more anterior parts of the small intestine, which are not so crowded, will be found greater the farther the worms are from that place of predilection and the rarer their occurrence. The worms will finally reach their normal size in samples taken from far forward. In the cecum when the concentration of trichinae proves to be high, the length of the worms, too, is somewhat less than normal, males reaching 1.0 to 1.3 mm and females 1.6 to 2.2 mm.

SUMMARY

Examination of the intestines of more than fifty guinea pigs, infected with known numbers of larvae of *Trichinella spiralis,* showed that a large majority of the adult worms had accumulated in the posterior sections of the intestine, i.e., at the posterior end of the small intestine, throughout the cecum, and at the beginning of the large intestine.

Localization is very clear in the small intestine, the concentration of trichinae being highest in the last few centimeters, and decreasing rapidly towards the anterior end. The lighter the infection given, the higher the percentage of adult worms seemed to be at the posterior end of the small intestine. Vice versa, a heavy infective dose caused enormous concentration of trichinae in the cecum and at the posterior end of the small intestine, as well as sparse distribution throughout the whole small intestine.

The longevity of the adult trichinae established in the guinea pig intestine proved to be much higher than is generally found in rats, rabbits, mice, dogs and other animals. The worms may live in the small intestines of guinea pigs for 30 to 37 days (in one case for 50 days) and in the cecum for 42 to 55 days. Because of the long life of the adults the production of young larvae is very considerable, beginning from the 6th to the 8th day after infection and finishing as a rule from the 30th to the 37th day after infection.

The length of mature trichinae, (males measure normally 1.4 to 1.6 mm and females 2.2 to 3.6 mm), may, in cases of extraordinary longevity, reach 2.0 to 2.2 mm for the males and 4.0 to 4.8 mm for the females.

In cases of very heavy infection the growth of the worms, although they were fully developed sexually, was greatly restricted at the posterior end of the small intestine, where the accumulation was highest. Many males measured only 0.6 to 0.8 mm and many females 1.1 to 1.6 mm.

Bibliography

McCoy, O. R. 1931 Immunity of rats to reinfection with *Trichinella spiralis*. Am. J. Hyg. **14**: 484–494.

————1932 Size of infection as an influence on the persistence of adult trichinae in rats. Science **75**: 364–365.

Matoff, K. 1937 Der Mechanismus der Altersimmunität des Hundes gegen die Trichinelleninfektion. Tierärztl. Rundschau **43**: 354–359 and 369–373.

Matoff, K. and Wapzarowa, M. 1937 Wieviel Jungtrichinellen kann eine weibliche Darmtrichinelle gebären? Z. Infektionskr. Haustiere **51**: 89–98.

STUDIES ON STRONGYLOIDES. III. THE FECUNDITY OF SINGLE *S. RATTI* OF HOMOGONIC ORIGIN

GEORGE L. GRAHAM*
Department of Animal and Plant Pathology, Rockefeller Institute for Medical Research, Princeton, N. J.

INTRODUCTION

In a previous article (Graham, 1938) attention was called to the persistent production of progeny of both direct and indirect development throughout the life span of the homogonically derived, singly established, *Strongyloides ratti* parasite in the laboratory rat host. In the course of this study a large body of data on the fecundity of this form was obtained. The fecundity of mass nematode infections has been frequently analyzed in terms of eggs and larvae per female worm, thus indicating that interest is attached to the reproductive performance of the individual parasite. The present study concerns single, fertile, nematode parasites, objectively established in their normal hosts.

MATERIALS AND METHODS

The materials and methods employed are the same as described earlier (Graham, 1936; 1938). Only progeny counts obtained from 24-hour fecal cultures which were incubated as collected, without crushing or mixing of the pellets, have been included in these data. It was found (Graham, 1938) that crushing and mixing of the pellets composing the cultures resulted in reduced yields of progeny.

The progeny counts have been employed analytically without reference to the type of progeny involved, except that cultures containing filariform larval progeny of the free-living generation were excluded. Such cultures were of infrequent occurrence. Only cultures containing one or more progeny were considered in Graham (1938). Cultures from which no progeny were isolated have been included in the present instance, provided they originated from a rat host containing a parasite which had produced progeny prior to, as well as subsequent to, the day on which each negative culture was collected. Of the 7,513 cultures employed, nearly 25 per cent were of this negative status.

THE FECUNDITY OF *S. ratti*

1. *General Considerations*

The necessity of observing "old" infections was responsible for a disproportionately large number of cultures examined in the earlier weeks

Received for publication, October 4, 1937.
* The writer gratefully acknowledges his indebtedness to Dr. Norman R. Stoll for criticism and suggestions during the course of these studies.

of the infections. Patency terminated within six months as a rule, with only small numbers of the single parasites remaining fecund for longer periods of time.

Progeny counts from 120 single *S. ratti* are involved. In addition to the 116 infections used and presented in detail in Graham (1938), there are two infections each from the 35th and 36th serial generations. From these 120 infections, 7,513 daily cultures have been obtained and the counts from these employed in this study. Progeny counts were reduced to a common base, i.e., time of infection, and grouped by weeks of parasitic age of the single females from which the offspring originated.

In the data presented by Graham (1938) it was reported that 92 infections with single *S. ratti* had originated serially from a single homogonic larva and that 24 had been extraneous, single, homogonic larva infections. Slight differences in the relative production of progeny of heterogonic development were observed between these two groups, but they did not indicate separate consideration except as an analytical precaution. Comparisons of these same groups with regard to their respective fecundity provided no evidence of dissimilarity and they are consequently analyzed as a single group.

2. *Median Weekly Progeny Obtained from Single* S. ratti

In figure 1, the median number of progeny (total of both direct and indirect) produced by 120 single *S. ratti* are shown, in relation to the duration of parasitism. These weekly values are seen to be comparatively low, but the period of maximum fecundity is clearly demonstrated. In weeks II and III it reached 17 progeny daily. After week III, the median began to descend almost as abruptly as it rose during the first two weeks of infection, and beyond week VI it never exceeded four progeny daily. In fact, for 47 of the 49 following weeks it did not exceed three.

The weekly median yields as graphed in figure 1 were not derived from equal numbers of daily progeny counts in each week. In each of the first 18 weeks, with the exception of week I, they are based on more than 200 daily progeny counts, weeks XIX–XXVI having between 100 and 200.

The addition of substantial blocks of new counts to the data from time to time has indicated that only occasionally does a shift of the median value occur. Then the change has been of only a minimal order. Such additions of new data in weeks where smaller numbers of daily cultures (10–40) were originally available effected some straightening of minor fluctuations in the curve. It would appear that 10–15 patent single *S. ratti* provide a sufficient number of counts, i.e., 70–100, for the establishment of a weekly median value of their daily fecundity at any stage of

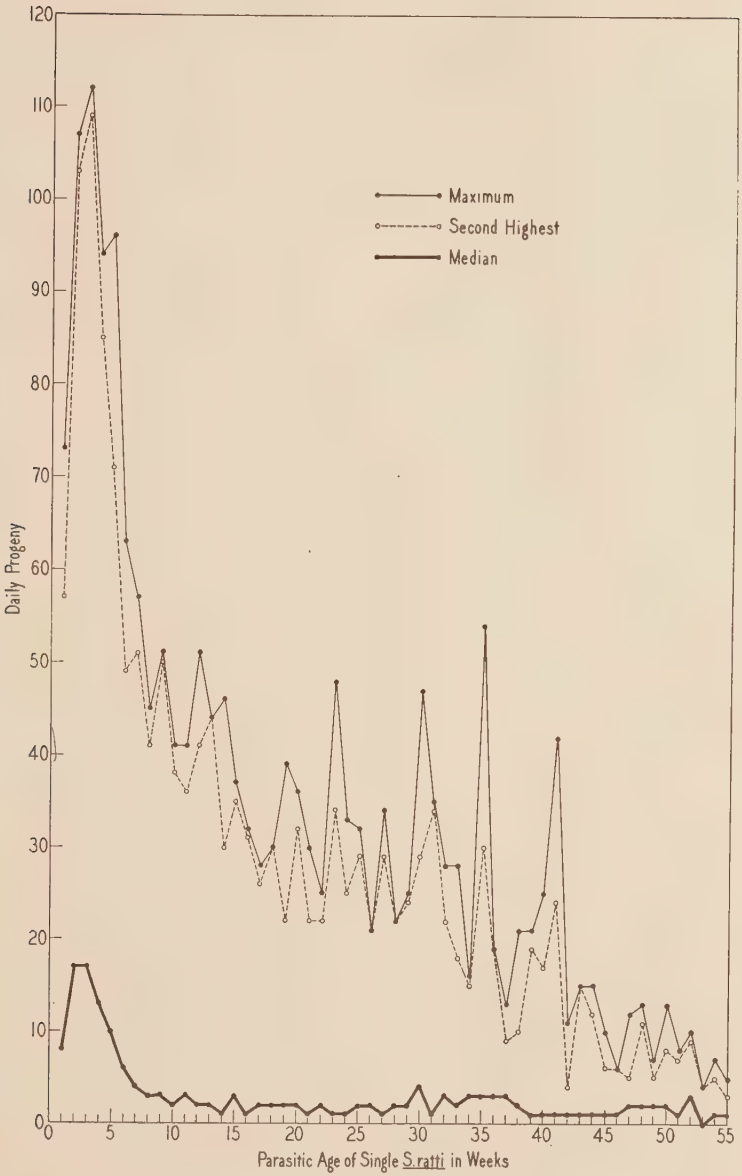


FIG. 1.—The median daily yield of progeny from 120 single, homogonically derived *S. ratti* parasites in the rat host, in consecutive weeks of age. 7,513 individual daily cultures secured over an age range of 55 weeks are involved. The maximum, as well as the second highest, daily yield in each week is also shown.

their reproductive life. This value is slightly refined but not materially changed by the utilization of additional infections.

Thus the median curve depicted in figure 1 effectively assays the reproductive performance of single, homogonically derived *S. ratti* under conditions presumably ideal for the manifestation of species characteristics and becomes an adequate expression of their fecundity. It delineates the rapid increase in progeny output which occurs in the early weeks of the infections and the subsequent and almost equally rapid decline in the progeny numbers which follows the period of maximum yield. Lastly, it emphasizes the tenacity of at least a few of the singly established parasites in persisting and remaining fecund, though at a very low level, for rather unusual lengths of time considering the minute size of these parasites.

By way of contrast to the median, and as an indication of the greatest variation which occurred in the distribution of the daily progeny numbers, curves of the highest and second highest daily counts in each week are also shown in figure 1. Maximum yields were obtained during weeks II and III. These declined rather sharply for the next five weeks, then more gradually. By week XV, the highest obtained yield had dropped to less than 40 progeny for any day, and subsequently exceeded this level on only four isolated occasions, viz., in weeks XXIII, XXX, XXXV, and XLI. In these four instances the maxima were respectively 48, 47, 54, and 42. As observable in the curve, they were evidently isolated accumulations of ova ascribable to either the parasite or the host, for in each of these weeks the second highest yield was notably lower and originated from infections other than those which produced the above mentioned maxima.

3. *Daily Number of Progeny Arranged in Yield Categories*

To supplement the picture of daily progeny yields described by the median, a grouping of the numbers of daily offspring has been used. Five arbitrary yield categories have been chosen, viz., daily counts yielding 0 (during patency), 1–10, 11–20, 21–40, and over 40 offspring from the cultures. In figure 2 are presented graphically the weekly percentage distributions in the five yield categories for the first 29 weeks of parasitism. For the remaining 26 weeks, they have been condensed into six consecutive groups on the time scale to provide more comparable numbers of cultures. All 7,513 progeny yields secured from 120 demonstrably patent single *S. ratti* infections are included.

Examination of figure 2 clearly shows the trend of the daily progeny output as the individual mother worms, from which they were derived, increased in age. Once patency was established, negative cultures rarely occurred in week I, most cultures yielding from one to ten offspring and only occasionally over 40. During weeks II and III, when the number

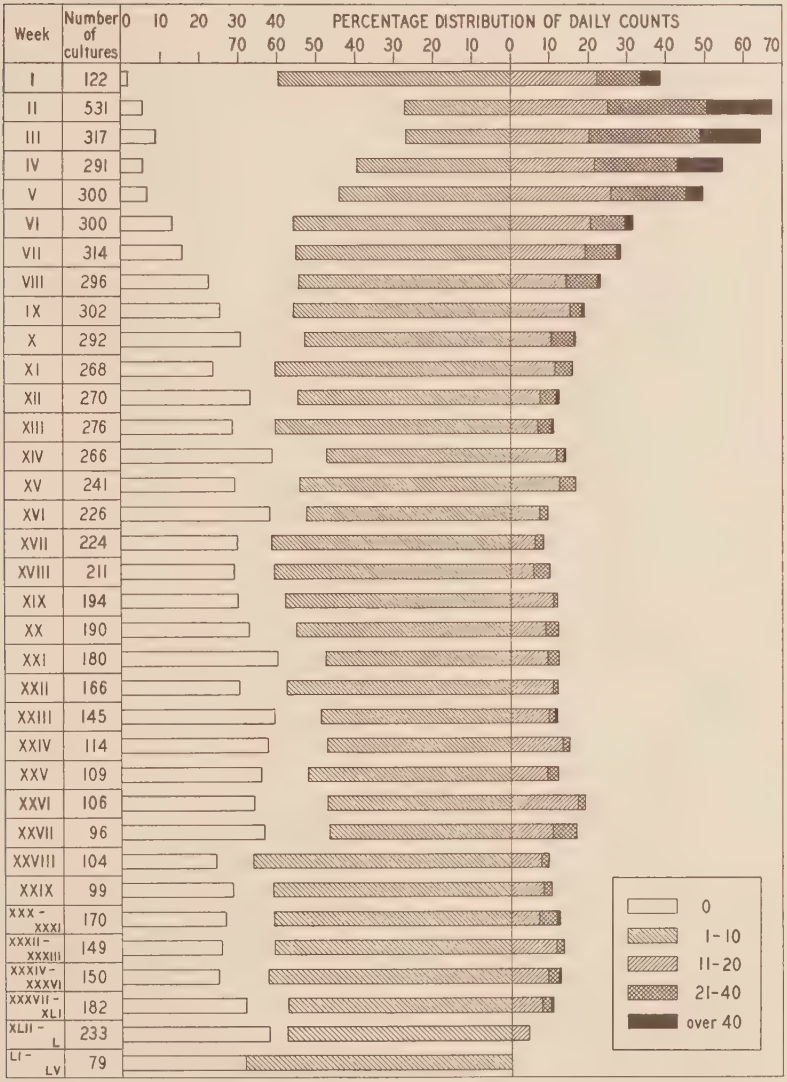


FIG. 2.—The percentage distribution of the numbers of offspring from 120 single *S. ratti* in five yield categories, viz., 0 during patency, 1–10, 11–20, 21–40, and over 40 progeny. The daily yields are shown by weeks for 29 weeks; beyond this point weeks are grouped to produce roughly comparable numbers of cultures observed.

of offspring increased to the maximum, there was a corresponding decrease in the 1–10 category as compared with week I; cultures yielding over 40 progeny were most frequent and negative counts infrequent.

In weeks IV and V, culture yields of 1–10 progeny occurred with increasing frequency, while there was a corresponding decline in the yields from the higher categories. The picture of fecundity of single *S. ratti* to this point was one of rapid increase after the onset of patency, with the period of maximum fecundity occurring in weeks II and III, and moderate, but obviously declining, fecundity in weeks IV and V, indicative of the lower productivity still to follow.

Beginning with week VI, days with no offspring occurred with increasing frequency. By week VIII, over 20 per cent of all counts were negative. While these negatives increased less rapidly thereafter, they approached and often exceeded a 30 per cent level during subsequent weeks. During this time, frequencies in the 1–10 category reached and fluctuated between the 50 and 60 per cent level, with frequencies in the higher yield categories gradually declining and reaching a level of approximately 15 per cent by week XI. During this period (weeks VI–XIV) only very small percentages of the cultures contained over 40 progeny and after this they were rarely encountered, although a culture with over 40 offspring occurred once as late as the 41st week after infection (fig. 1).

The large number of negative cultures which were encountered during the patent period after week V was a premonitory sign that many infections were approaching the post-patent period. As a rule, before final positive cultures were obtained, progeny reached very low numbers. These low yield positives were frequently interspersed between negative days, negative periods of a week's duration being not at all uncommon and periods of shorter negativity numerous. Occasionally, consecutive daily cultures would be negative for two weeks or more before positive cultures intervened to prolong patency. Only on rare occasions did a sequence of positive cultures terminate abruptly. Usually, the end of patency was forecast for some weeks or even months by the increasing frequency with which days with no offspring occurred.

4. *Progeny from Representative Single* S. ratti *Parasites*

Daily yields of progeny from representative individual parasites are shown in figures 3a and 3b. Three single *S. ratti,* sisters, of the 18th serial generation, parasitizing three female rats, litter mates, are concerned. The yields are typical of those obtained from other single worms, although instances of greater, as well as lower, individual maximum yields have occurred. These three highly comparable cases, in rat hosts nos. 318, 319, and 322, were selected for graphic presentation because of

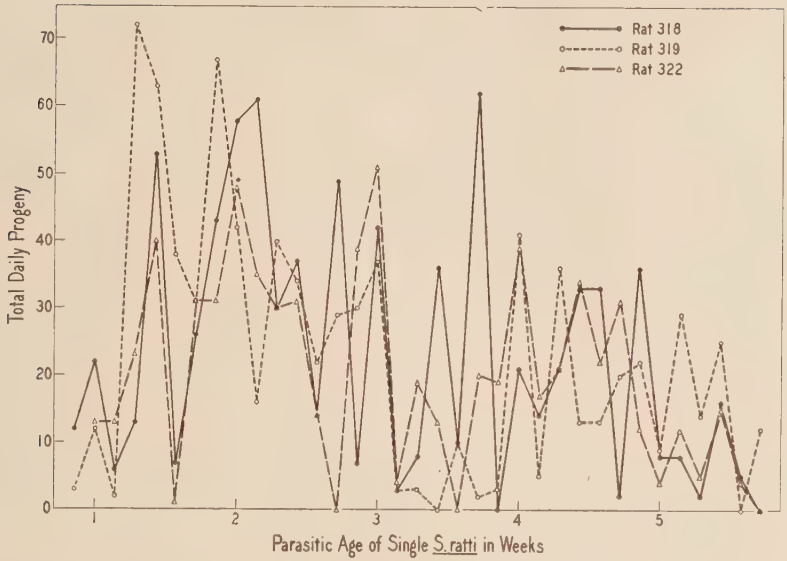


FIG. 3a.—The numbers of progeny produced daily by three *S. ratti,* sisters, of the 18th serial generation, each parasitizing a female rat, sisters, during the first 40 days of parasitism.

the high degree of host and parasite homogeneity, and the fact that an uninterrupted series of daily progeny counts was available from the onset of patency until 40 days after they had each been exposed to a single larva.

FIG. 3b.—The data of fig. 3a arranged as 3-day moving averages.

In figure 3a the characteristic day to day variations of the progeny numbers are depicted. In spite of the fluctuations in the daily yields, the period of greatest fecundity in week II–V and the ensuing decline are discernible. In figure 3b, the same data are shown smoothed as three-day moving averages.

Since there is no question with regard to the number of worms involved in these cases, the daily progeny numbers are a reflection of the fecundity of one worm as it is modified by inherent or environmental factors. It is difficult to dissociate the rôle of the parasite and that of the host in producing the observed fluctuations. Obviously, there is a lag between the time of oviposition and the time when eggs, or the larvae from them, pass from the host in the fecal pellets. Any factors which contribute to this lag, such as variability in the time required for eggs and larvae to emerge from the mucosa into the lumen of the gut, and irregularity in the movement of material through the gut, may well account for

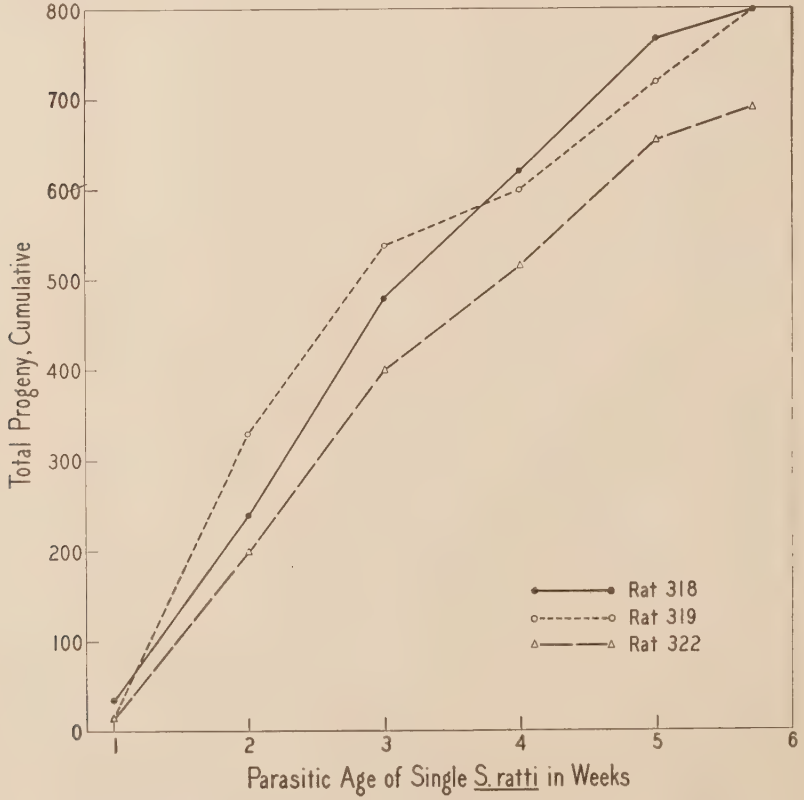


FIG. 4.—The cumulative number of progeny produced by three single *S. ratti* during 40 days of parasitism. Their progeny are given in fig. 3a as numbers observed daily, and in fig. 3b the same data are graphed as 3-day moving averages.

a great deal of the variability in the daily yields. That factors independent of the parasite may be involved is suggested in figure 3a since large numbers of progeny on one day are frequently followed on the next by low yields. Possibly this is correlated with some feeding cycle of the hosts.

Another comparison of the progeny output from these three single *S. ratti* infections is given in cumulative total progeny curves, in figure 4. A striking parallelism in the fecundity of the three parasites is clear. At the end of 40 days of parasitism, two of the nematodes (those in rats nos. 318 and 319) had produced exactly the same number of progeny, 798 having been enumerated from each. The total from no. 322 over the same period was 691.

DISCUSSION

The number of single larva infections established and the number of daily progeny counts which they provided, constitute an adequate basis for obtaining a clear view of the fecundity of essentially one strain of *S. ratti* in single parasitism. The composite picture of progeny production during the entire reproductive life of single *S. ratti* which has been presented overcomes to a satisfactory degree the daily fluctuation of the progeny numbers. Although the day to day variations in the numbers of progeny shed by individual infections varied, sometimes over a rather wide range as was shown in figure 3a, the limits of that variation were not extreme when one recognizes that the offspring of a *single* worm were thus enumerated.

The 7,513 daily progeny counts which were employed in determining the median curve (fig. 1) make this value a highly stable one, particularly in the early months of the infections. Consequently, it seems possible to consider the weekly median yields as a conservative measure of the fecundity of a single *S. ratti* parasite subjected to a minimum of adverse influences such as immunity, "physiological crowding," or other deleterious host reactions against an invading organism.

While the various techniques of collecting the fecal pellets, culturing, isolating and counting the progeny no doubt entailed some losses, it is believed that such losses represented but a small fraction of the total egg production of the single female. It is the writer's conviction that no other combination of technical procedures would be as efficient if applied to an infection consisting of a single *S. ratti* parasite. On a comparative basis, the data as gathered have high validity. Sheldon (1937) found direct counting of *S. ratti* progeny from screened and concentrated feces unsatisfactory, "because large numbers of larvae became disintegrated beyond recognition."

One source of loss which is certainly negligible with these single *S. ratti* infections is destruction of numerous larvae by digestion following coprophagy by the host. While rats under poor nutritive conditions will eat their own feces with avidity, the rats used in the present studies had constant access to a well balanced and nutritionally adequate dry mash ration. Since they were held in screen-bottomed cages, pellets seldom failed to fall promptly through into the water of the collecting pans below. Thus opportunity for coprophagy was reduced to a minimum, if it occurred at all. Autopsies on a number of rats harboring heavy stock infections of *S. ratti* never revealed dead and degenerating larvae, although many adult females and numerous unhatched eggs and motile rhabditiform larvae were observed in scrapings from the intestinal mucosa.

Patency usually began on the 6th and 7th day after infection and less frequently on the first or subsequent days of week II (Graham, 1936). Infections have been observed to become patent as early as the 5th day and as late as the 13th day after infection. Sheldon (1937), working with mass infections of *S. ratti,* observed larvae in cultures collected one day after infection and the rats "patent" again on the 3rd day. Four other groups were "patent" on days 3, 3, 4, and 6 after infection, respectively, with the first examination after infection being made, apparently, on the 3rd day. In explanation of this assumed difference in the length of the prepatent period between his mass infections and those reported by Graham (1936), Sheldon, referring to the small output of progeny early in the infection, said, "During this period the daily larval output of a single parasitic female is conceivably so small that any slight adverse variations in cultural conditions may render them indiscernible." The question may be asked as to whether there is any real basis for comparison. Since Sheldon observed animals to be ostensibly "patent" one day after infection, the question is raised as to whether adequate differentiation can be made on the 3rd day after infection between filariform larvae of the infecting inoculum which failed to infect and filariform larval progeny from those which succeeded in establishing themselves. If adequate differentiation has not been made (and it has not been specified), there is, of course, no comparison between the two cases.

In this connection it is interesting to note Scott's (1928) observation of *Ancylostoma caninum,* morphologically "identical with infective larvae," which were recovered from the intestinal tract of rats, cats, and dogs on the 21st, 44th, and 33rd day respectively after these animals had been given infective larvae. If there is any analogy between mass infections of *A. caninum* and *S. ratti* in the rat, then the recovery of aborted larvae in the feces is a dubious indicator of patency for the latter nematode.

SUMMARY

1. Over 7,500 progeny counts, secured from 120 single, homogonically derived *S. ratti* and covering a parasitic age range of 55 weeks, have been employed in determining the fecundity of this nematode. The daily progeny expressed as a median value on a weekly basis adequately delineates the reproductive performance of these single worms. Progeny production was at a maximum in weeks II and III (median = 17), after which the median progeny yields declined rapidly to a low level which was maintained over the indicated age range.

2. The percentage distribution of the progeny counts in five yield categories, viz., 0, 1–10, 11–20, 21–40, and over 40 progeny, is presented in relation to the age of the singly established mother worms. The characteristic early increase and subsequent decrease in fecundity was followed by an extended period of gradually declining productivity.

3. The daily progeny production of three representative sister *S. ratti*, each parasitizing sister rat hosts, is presented, and the daily variations in the progeny output, as well as the striking similarity in the total output, shown.

BIBLIOGRAPHY

GRAHAM, GEORGE L. 1936 Studies on Strongyloides I. *S. ratti* in parasitic series, each generation in the rat established with a single homogonic larva. Am. J. Hyg. **24**: 71–87.

————1938 Studies on Strongyloides II. Homogonic and heterogonic progeny of the single, homogonically derived *S. ratti* parasite. Am. J. Hyg. **27**: 221–234.

SCOTT, J. A. 1928 An experimental study of the development of *Ancylostoma caninum* in normal and abnormal hosts. Am. J. Hyg. **8**: 158–204.

SHELDON, A. J. 1937 Some experimental studies on *Strongyloides ratti*. Am. J. Hyg. **25**: 39–52.

BRACHYLAEMUS PEROMYSCI N. SP. (TREMATODA) FROM THE DEER MOUSE

BRUCE D. REYNOLDS
University of Virginia

Specimens on which this description is based were obtained from the small intestine of the deer mouse, *Peromyscus leucopus leucopus* (Rafinesque). Only one trematode was obtained from each of two animals of twenty-five examined. Both of these specimens were essentially alike and differed sufficiently from existing species of *Brachylaemus* to necessitate establishing a new specific name for them. The name *Brachylaemus peromysci* n. sp. is proposed.

Brachylaemus peromysci n. sp.

(Fig. 1)

Specific diagnosis: Brachylaemus, Family BRACHYLAEMIDAE. Body flattened ventrally, convex dorsally, ends rounded; length of sexually mature specimens 2.8 mm, width 0.51 mm. Acetabulum divides body 1 : 6. Cuticle weakly spinose. Oral sucker subterminal, 0.16 × 0.19 mm. Acetabulum 0.18 × 0.19 mm, hence slightly larger than oral sucker. Pharynx 0.09 × 0.11 mm. Esophagus very short. Intestinal caeca arise immediately posterior to pharynx and extend to near posterior extremity of body. Genital pore ventral and median near front margin of anterior testis. Gonads intercecal, contiguous, tandem, ovary between testes, in posterior fourth of body. Anterior testis 0.18 × 0.20 mm, posterior testis 0.20 × 0.22 mm. Vasa efferentia emerge from anterior margins of testes and apparently enter seminal vesicle separately. Seminal vesicle runs forward in sinuous course from front margin of anterior testis for approximately 0.12 mm, then bends ventrad and posteriad to join the small muscular penis which runs posterio-ventrad to enter genital atrium from left side. Ovary 0.21 × 0.25 mm located dorsal to Mehlis' gland. Oviduct arises from dorso-posterior surface of ovary and proceeds ventro-anteriad, giving off Laurer's canal which opens on median dorsal surface above ovary. Seminal receptacle 0.03 mm in diameter is located near origin of Laurer's canal. Duct from yolk reservoir enters oviduct between seminal receptacle and Mehlis' gland. From Mehlis' gland uterus runs forward ventrally in numerous folds to region of pharynx where it bends back and returns posteriorly on dorsal side in somewhat larger loops until it nears genital pore. From here metraterm proceeds ventrally to enter genital atrium from right. Numerous uterine coils practically fill intervitellarian space and extend from gonads to pharynx. Vitellaria extend in finely lobulated masses along each side from near genital pore to posterior half of acetabulum; from near the posterior ends of each, a yolk duct passes marginal to anterior testis and ovary to yolk reservoir lying just posterior to ovary. Eggs in preserved specimens average 17 × 28 μ. Excretory bladder cone-shaped, short, intercecal.

Host: Peromyscus leucopus leucopus.

Habitat: Small intestine.

Locality: Charlottesville, Virginia, U. S. A.

Type: U. S. Nat. Mus. No. 9125. Paratype also in U. S. Nat. Mus.

B. peromysci is very similar to *B. virginianus* (Dickerson) Krull, 1934, described from this laboratory in 1930, from *Didelphis virginiana.*

Received for publication, October 7, 1937.

In order to check on the various measurements and to compare the morphological features of the flukes found in the deer mouse and opossum, four opossums from the same general locality were examined. All of these were found to be infected with a total of 80 specimens of *B. virginianus*. These were prepared by the same methods as had been used for those found in the deer mouse as follows: Fixed in Beauchamp's acetoformol-alcohol under slight pressure and stained in alum cochineal-hematoxylin as recommended by Reynolds (1936).

OBSERVATIONS

A comparative study of the two forms fixed and stained in the same manner showed that the specimens from the deer mouse were slightly larger, both longer and broader, while both suckers were smaller. In *B. virginianus* the acetabulum divides the body in the ratio of 1:3.5, while in *B. peromysci* this ratio is 1:6. In the latter the ovary is larger than the average for the testes (232:200), while in *B. virginianus* the ovary is much smaller than the average for the testes (150:253). Furthermore, the eggs of *B. peromysci* are shorter and rounder, 17×28 microns as compared with 16×32 microns. These differences are given in Table 1. The general structure of *B. peromysci* is shown in Figure 1.

TABLE 1.—*Measurements in microns of* B. virginianus *as given by Dickerson, Krull, and present paper, and of* B. peromysci

	B. virginianus Dickerson, 1930	*B. virginianus* Krull, 1935	*B. virginianus* this paper	*B. peromysci* this paper
Average length	1945	2600	2555	2800
Average width	368	315	480	510
Oral sucker	259×285	227×208	249×213	190×160
Acetabulum	167×198	178×203	202×225	180×190
Pharynx	127×109	106	124×123	110× 90
Acetabulum divides body into	1:3	?	1:3.5	1:6
Anterior testis	186	208	228	190
Posterior testis	177	226	278	210
Ovary	91	122×113	123×178	214×250
Eggs	16×31	20×35	16×32	17×28

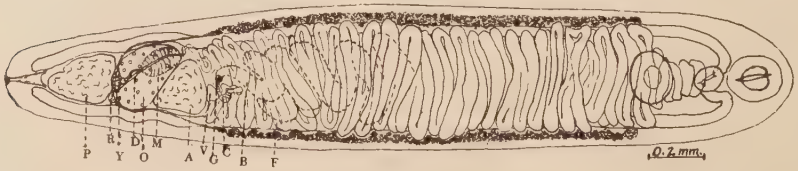


FIG. 1.—Ventral view of *B. peromysci*. A, anterior testis; B, descending stem of uterus; C, cirrus; D, oviduct; F, ascending stem of uterus; G, genital pore; M, Mehlis' gland; O, ovary; P, posterior testis; R, seminal receptacle; V, seminal vesicle; Y, yolk reservoir.

DISCUSSION

According to Dollfus (1935) two species of *Brachylaemus* have been reported from rodents in Europe and northern Africa, namely, *B. recurvus* Dujardin, 1845 = (*Heterolope eaquans* Looss, *D.* (*Br.*) *recurvuum* Duj., *Dist. musculi* Rud. (?)) and *B. advena* Duj., 1843 = (*D.* (*Br.*) *migrans* Duj.).

Joyeux and Foley (1930) reported a trematode from *Meriones shawi shawi* in northern Algiers which they identified as *B. recurvus* Duj., 1845. There are certain features of *B. recurvus* which are similar to the condition found in *B. peromysci,* viz., the ventral sucker is larger than the oral sucker and the dimensions of the eggs are about the same. By referring to Table 2 it will be seen, however, that the differences are

TABLE 2.—*Dimensions in microns of certain features of* B. recurvus *and* B. peromysci

	Length	Width	Oral sucker	Ventral sucker	Pharynx	Eggs
B. recurvus	up to 4000	700–1000	320	375	190	25 × 16
B. peromysci ..	2800	510	175	185	100	28 × 17

great enough to warrant considering them as different species. Whereas the dimensions shown in this table are of a corresponding magnitude, i.e. the larger fluke has larger parts, with exception of the eggs, these proportions are not brought into agreement by the application of a constant integer. Furthermore, a comparison of large and small specimens of a given species shows a greater constancy in the size of the suckers than exists in the dimensions of the organisms themselves. This is especially true of the pharynx.

Krull (1935) attempted to infect various mammals other than the opossum with metacercariae of *B. virginianus.* This was without success except that they did survive for two days in the white rat. In later experiments (but published earlier, 1934) he was able to infect dog, cat, chicken and white rat by feeding them metacercariae of *B. virginianus.* In these experiments Krull noted a difference in the rate of maturity and of growth after maturity in the different experimentally infected hosts. In all cases, however, the size was approximately the same or less than in the opossum. If dimensional structural differences appeared they are not mentioned. The writer agrees with Krull that host differences are not valid for establishing different species.

SUMMARY

A new species of *Brachylaemus, B. peromysci,* is described from the deer mouse, *Peromyscus leucopus leucopus.* It differs from *B. virginianus* in the opossum, the only other recognized species that has been reported as occurring in the state of Virginia, in the actual and relative

sizes of suckers, position of acetabulum, size of ovary, dimensions of eggs and in other minor details. It differs from *B. recurvus,* reported in rodents from Europe and northern Africa, in size of body and in the relative size of important structures.

Bibliography

Dickerson, L. M. 1930 A new variety of *Harmostomum opisthotrias* from the North American opossum, *Didelphys virginiana,* with a discussion of its possible bearing on the origin of its host. Parasitology **22**: 37–46.

Dollfus, R. P. 1935 Sur quelques *Brachylæmus* de la faune Française récoltés principalment à Richlieu (Indre-et-Loire). Ann. Parasitol. **13**: 52–79.

Joyeux, Ch. and H. Foley. 1930 Les helminthes de *Meriones shawi shawi* Rozet dans de nord de l'Algérie. Bull. Soc. Zool. France **55**: 353–374.

Krull, W. H. 1934 New experimental hosts for *Brachylaemus virginiana* (Dickerson) Krull. J. Wash. Acad. Sc. **24**: 483–485.

————1935 Some observations on the life history of *Brachylaemus virginiana* (Dickerson) Krull, 1934. Tr. Am. Micr. Soc. **54**: 118–134.

Reynolds, B. D. 1936 Alum cochineal hematoxylin stain. Stain Techn. **11**: 166–167.

SOME NOTES ON SARCOSPORIDIA IN VIRGINIA[1]

I. D. Wilson and Ruth McDonald[2]

Biology Department, Virginia Polytechnic Institute

In a brief study of sarcosporidiosis in Virginia an examination was made of the heart muscle of 35 cows, 29 calves, 27 sheep, and one horse. There was no visible evidence of the presence of cysts in any of the tissues upon gross examination. The material was fixed in Bouin's solution, embedded, sectioned, and stained with haematoxylin and eosin. In 30 out of 34 cow hearts cysts were found measuring, on the average, 74 by 252 μ. In sections cut 4 μ thick there was an average of three cysts to one eighth of a square inch of tissue, the highest number being 18. The muscle from the tongue of one cow contained four very small cysts to an eighth of a square inch, which were located in both the longitudinal and transverse muscle fibers.

All of these cysts had the same general appearance. Each was seen embedded in a single muscle fiber. There was no thick envelope surrounding any of the cysts. In one or two cases the division into compartments was very clear. In others the cyst seemed to be undivided. There were visible septa between the compartments in a few sections. Some spores appeared to be lined along the septa and to lie crosswise in direction rather than longitudinally. Others in the center of the compartments had no definite axial placement. The muscle surrounding the cyst was in every case normal in appearance. The spores within the cyst were banana shaped, about ten by three μ in size, with a large central comparatively clear space, presumably the nucleus. The cytoplasm at one end stained dark blue with the haematoxylin and light pink with the eosin. These cysts and their contents appeared very different, in the size of the cyst itself, the enveloping membrane, and the size and shape of the spores, from those described by Besnoit and Robin (1912) which were found in the skin.

Of the sections from the 27 sheep hearts only eight were found to be infected. Not more than three cysts were found in any one of the sections. The cysts measured 102 by 52 μ. They resembled closely those found in the cattle hearts, except that there was no visible division into compartments. The banana shaped spores contained within were packed quite closely with no regular placement and measured 7 by 3 μ.

Received for publication, October 16, 1937.

[1] Departmental contribution No. 41.

[2] The authors wish to express their appreciation to Dr. Henry Marshall, Inspector in Charge, Bureau of Animal Industry, U. S. Department of Agriculture, Richmond, Va., from whom specimens of heart muscle were obtained.

A piece of muscle from the neck of a horse was sectioned. Cysts were found which had the same general appearance and measurements as the others studied.

In the heart muscle from 29 calves, 6 to 8 weeks old, no cysts were recognized. This confirms the statement of other authors that very young animals which have not been out on pasture are not infected and also gives credence to the theory that some insect may be the intermediate host, a theory brought to light by Darling (1915), but which Scott (1920) was unable to demonstrate.

The parasites which were studied were quite different from those found in the muscle of a rabbit and the pectoral muscle of a duck. This may be because we may have been working with young cysts which would account for the small size, and the lack of a demonstrable layered membrane. However, since these specimens were obtained in January, February, and March, and Scott (1918) has shown a definite seasonal infection which occurs between May and October, it seems there should have been a majority of mature cysts rather than young ones.

SUMMARY

Cattle in Virginia appear to be commonly infected with sarcosporidia as we failed to find infection in only four out of 35 cattle examined. Sheep seem not so heavily infected, as in only eight out of 27 sections were cysts found, however, it is possible that if the animals had been older more cysts would have been found. No cysts were found in sections from calf hearts.

All parasites studied had the same general appearance in cyst and cyst contents. No three layered membrane was found, and in only a few cases in cattle were the trabeculae seen dividing the cyst into compartments.

Our examinations were superificial as only a small section of the heart from each animal was studied. However, they serve as an index as to the prevalence of this parasite in cattle and sheep in the state of Virginia.

Bibliography

Besnoit, C. and Robin, V. 1912 Cutaneous sarcosporidiosis in a cow. Rev. Vet. (Toulouse) **37**: 649–663.

Darling, S. T. 1915 Sarcosporidia encountered in Panama. J. Parasitol. **1**: 113–120.

Scott, J. W. 1918 Progress report on *Sarcosystis tenella*. II. Seasonal infection. Ann. Rep. Wyo. Agric. Exper. Sta. pp. 96–116.

———1920 Notes and experiments on *Sarcocystis tenella* Railliet. III. Is *Sarcocystis tenella* an aberrant form of one of the cnidosporidia of insects? J. Parasitol. **6**: 157–166.

A NEW SPECIES OF *TRICHODINA* (CILIATA) FROM THE URINARY TRACT OF THE MUSKALONGE, WITH A REPARTITION OF THE GENUS.*

JUSTUS F. MUELLER
New York State College of Forestry, Syracuse, N. Y.

In 1932 the author described *Trichodina renicola* from the urinary tract of the pickerel, *Esox niger,* in Oneida Lake. This was the first record of a *Trichodina* from the urinary bladder of a fish. On numerous subsequent occasions the urinary tract of *Esox lucius* from various localities was examined for related ciliates, but with negative results. An added interest therefore attaches to the discovery of a similar parasite in the urinary passages of the muskalonge, *Esox masquinongy,* of Chautauqua Lake, N. Y. This parasite was present in all adult muskalonge examined.

METHODS

Urinary bladders and kidneys of infected fish were removed and fixed in formalin or Bouin's fluid and later sectioned. The ciliates were also studied alive, and in the form of dried film or smear preparations. In such case the bladder was opened and its contents smeared over several slides, with or without dilution with a little water, and allowed to dry. Smears were later fixed in Bouin's fluid or 100 per cent alcohol and stained in Ehrlich's hematoxylin and eosin. Bladders that had been fixed in Bouin's were later opened and their *Trichodina* stained and mounted as whole mounts. These preparations were useful in preserving the *Trichodina* in their natural shape, whereas dried preparations resulted in flattening of the body and distortion of the soft parts. Dried film preparations are, however, ideal for studying the details of the skeletal ring, as in these slides the ring flattens out and lies in the plane of the field of vision. If sections or dried film preparations are stained for a prolonged period, 12 hours or more, in alcoholic eosin, the skeletal ring stains brilliantly. Unfortunately, reagents or facilities for fixing material for refined cytological study were not available at Chautauqua Lake. Such study was therefore postponed.

Received for publication, November 11, 1937.

* The material forming the basis of the present paper was gathered during the summer of 1937 while the author was serving as parasitologist with the Biological Survey of the New York State Conservation Department, under the direction of Dr. Emmeline Moore. Acknowledgment and thanks are herewith extended to Dr. Moore for support during the course of this study and for permission to publish the present paper.

DESCRIPTION OF THE CILIATE

The animal is high and conical in form, with a deeply concave posterior sucking disk. In 14 individuals taken at random the skeletal ring contained the following number of denticles: 37, 36, 38, 40, 37, 37, 38, 37, 39, 37, 40, 39, 38, 36, with the mode at 37, but with extremes of 36 and 40. The diameter of the skeletal ring, measured from the outer edge of the striated ring, in dried film preparations, for 13 individuals taken at random was as follows: 71, 64, 62, 64, 67, 66, 66, 69, 71, 75, 73, 73 and 62 μ. Variation is between extremes of 62 and 75 μ.

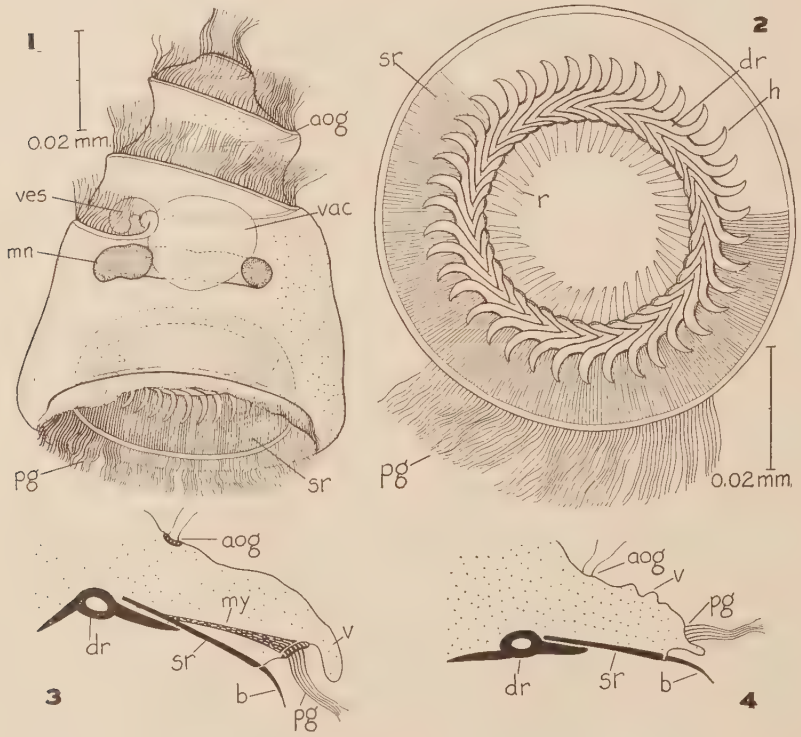


Fig. 1. Drawing of *Vauchomia nephritica.*

Fig. 2. Drawing of sucking disk in a dried film preparation. Posterior girdle of cilia only partly represented.

Fig. 3. Diagrammatic cross section of edge of sucking disk in *Vauchomia.*

Fig. 4. Diagrammatic cross section of edge of sucking disk in *Trichodina pediculus.*

Abbreviations: aog—adoral groove, b—border of sucking disk, dr—denticulate ring, h—hook, mn—macronucleus, my—myoneme, pg—posterior girdle of cilia, r—ray, sr—striated ring, v—velum, vac—vacuole, ves—vestibulum.

There are approximately 10 rays in the striated ring between each two hooks of the denticulate ring. The striated ring is of compound

nature, consisting of an anterior ring of soft rays, and a posterior ring of hyaline or hard rays. The anterior soft rays arise very close to the inner ends of the hard rays, but the two series are separate, and the ring of soft rays may part in places from the posterior ring in drying smear preparations. The soft rays terminate in a well defined groove forming the base of origin of the posterior girdle of cilia, whereas the hyaline rays terminate outwardly in contact with the thin membranous edge of the sucking disk. There appear to be as many rays in the anterior ring as in the posterior, but the two sets are not always parallel, and may lie at a slight angle to each other in the different levels. Unlike the stiff mechanical arrangement of the hyaline rays, the soft rays are frequently wavy and hence irregular in spacing.

Anterior rays were also described in the earlier account of *Trichodina renicola,* but were incorrectly interpreted as branches of the hard rays, and as being similar to these in nature, a view that has now been disproved. For lack of detailed cytological study I am unable as yet to arrive at definite conclusions, but it now appears most likely that these soft rays are a system of myonemes connected with the posterior girdle of cilia. The presence of such myonemes would account for the pronounced motility of the sucking disk in the present species and in *T. renicola.* In free swimming individuals of both species the sucking disk is observed to undergo frequent changes of form, and is often flexed and folded like a pocketbook. It would be difficult to account for such activity on the part of the posterior region of the body without the presence of a system of myonemes associated with the sucking disk. In any case the compound nature of the striated ring in the present species and in *T. renicola* separates these species from other forms in which only the hyaline rays are present.

The adoral spiral of cilia makes three complete turns of the anterior conical body region and descends into the vestibulum and gullet. The contractile vacuole opens into the vestibulum through a short canal as in *T. pediculus.* The macronucleus is in the form of a horseshoe with its ends lying on either side of the gullet, and is often observed in various stages of fragmentation, probably representing phases in the process of endomixis. The velum is well developed and projects backward over the posterior girdle of cilia.

LOCATION IN HOST AND PATHOLOGY

The parasites are abundant in the urinary bladder of the host, adhering to its walls by their powerful sucking disks. They also ascend the ureters as far forward as the lumen will permit, passing by this means into at least the posterior third of the kidney. Their size apparently does not allow them to enter any of the branches of the ureter. In infected

ureters the epithelium appears loosened in places and many cells have sloughed away into the lumen. In the bladder a large amount of such cellular debris can be seen along with the parasites. Apparently the ciliate causes an irritation by the action of its sucking disk which results in an extensive desquamation of cells from the walls of the urinary passages. The kidney tissue proper appears normal. No information is available as to the general effect of the parasite upon its host, since all adult muskalonge examined were found to be infected, and hence a standard of comparison was lacking.

TRANSMISSION

The mode of transmission is unknown. When removed from the urinary tract of the host into lake water the parasites live for an hour or longer. Placed in a glass dish they attach to the bottom so firmly that they cannot be readily dislodged with a stream of water blown from a pipette. Since encystment in this genus is unknown, it seems probable that the parasite must at times leave the host and pass into the lake water where it either finds a new host or perishes. Probably small numbers of *Trichodina* are constantly shed in the urine discharged from infected fishes. The most likely time for transmission to occur would be at the time of spawning when numbers of muskalonge are thrown temporarily into close proximity.

Water from Chautauqua Lake was pumped more or less continuously during the summer into the muskalonge rearing pond at the Chautauqua Hatchery at Bemus Point. After about two months rearing the young muskalonge in this pond had reached a length of from 6 to 8 inches and were returned to the lake. Examination of a sample of these fish showed them to be free from *Trichodina*. The result can only be accounted for on the grounds that the ciliate was not present in the water pumped from the lake into the pond. It is probable therefore that this ciliate does not occur widely distributed in the plankton.

Possibly when infection is first acquired there is an acute reaction, with effects not observed in older infected fish which may be regarded as having reached a chronic stage. Experiments on the transmission of the parasite under controlled conditions would have an added value in disclosing the ill effects, if any, upon the host during the initial stages of infection.

REPARTITION OF THE GENUS TRICHODINA

The members of the genus *Trichodina* fall naturally into two distinct groups. The first of these is represented by *Trichodina pediculus* in which the adoral spiral is limited to one complete turn of the body before descending into the vestibulum. In this group also the striated ring of

the sucking disk is simple, consisting of the posterior, or hyaline rays only. In the second group the adoral spiral of cilia performs from two to three complete turns of the body before descending into the vestibulum. The striated ring is compound, consisting of an anterior ring of soft rays (myonemes) in contact with the posterior girdle of cilia, and a posterior ring of hyaline rays in contact with the membranous border of the sucking disk. A further difference is found in the fact that in the form from the muskalonge a dense band of protoplasm forms the base of both the adoral spiral and the posterior girdle of cilia, whereas in *T. pediculus* this is lacking. Probably this dense band is also a myoneme, and its action opposes that of the radial myonemes.

The first of these groups is composed of species dwelling on the external surface of various aquatic animals—fish, amphibia, hydra, etc. The second group comprises forms found in the urinary bladder of fishes and amphibia. Because this second group is well characterized it is proposed to recognize it as a new genus.

Vauchomia n. g.

Generic diagnosis: Peritrichous ciliates, close to *Trichodina,* having an adoral spiral of cilia which performs two to three complete turns of the body. The striated ring is compound, consisting of a posterior ring of hyaline rays, and an anterior ring of soft rays (myonemes). The two rings are fused at their inner edges. Outwardly the soft rays fuse with the groove of the posterior girdle of cilia, while the hyaline rays are in contact with the membranous border of the sucking disk.

Type species: Vauchomia nephritica n. sp., from the urinary tract of the muskalonge in Chautauqua Lake, N. Y.

An additional species belonging to the new genus is *V. renicola* (Mueller, 1931), syn. *Trichodina renicola* Mueller, 1932, syn. *Cyclochaeta renicola* Mueller, 1931, from the urinary tract of *Esox niger. Trichodina okajimae* Ibara, 1931, from the urinary bladder of the Japanese salamander, and *Trichodina urinicola* Fulton, 1923, are *species inquirendae.* Although Ibara states that the adoral spiral makes one complete turn, he plainly figures an individual in which the adoral spiral performs more than two complete turns. If this figure is correct his species must be transferred to the genus *Vauchomia.* Fulton figures the adoral spiral of *Trichodina urinicola* as making one complete turn. If this is correct his species remains in the genus *Trichodina.* The validity of this position is questioned here only because of its habitat. A restudy of these forms would be desirable.

It is possible that a new genus may also be indicated for the species *Trichodina myakkae* Mueller, 1937, first reported from the bass in Florida, and during the summer of 1937 also found on carp and suckers in Chautauqua Lake. This form is characterized by the absence of rays on the denticulate ring, by the fact that the hooks are straight instead of

curved, and by the character of the adoral spiral which performs only half a turn of the body, thus being much shorter than in any other form. However, as a conservative course, no action is taken on this form at the present time.

With the above new genus there are now four easily distinguishable genera in the family URCEOLARIIDAE of Stein, which may be separated as follows:

1. Genus *Urceolaria:* With a ring of cirri anterior to the posterior girdle of cilia, and without hooks on the denticulate ring.

2. Genus *Cyclochaeta:* Like *Urceolaria,* but with hooks on the denticulate ring.

3. Genus *Trichodina:* Like *Cyclochaeta* but without cirri. Adoral spiral performs approximately one complete turn of the body.

4. Genus *Vauchomia:* Like *Trichodina* but the adoral spiral performs from two to three complete turns of the body.

COMPARISON OF V. RENICOLA AND V. NEPHRITICA

The two ciliates are roughly similar in size, but widely different in shape. *V. renicola* has the form of a small hemisphere, whereas *V. nephritica* has a high conical form. Because of the difference in shape of body the adoral spiral of cilia is much more conspicuous in *nephritica* than in *renicola. V. renicola* has about 56 denticles in the segmented ring, whereas *nephritica* has only about 36 or 37. The hooks and rays are more developed in *renicola* than in *nephritica.*

SUMMARY

1. A new species of ciliate, *Vauchomia nephritica,* close to *Trichodina* is described from the urinary bladder and kidneys of the muskalonge in Chautauqua Lake, N. Y.

2. The parasite appears to cause some desquamation of the epithelium of the urinary tract but no other effect was observed in infected adult fish.

3. The mode of transmission is unknown.

4. The genus *Trichodina* is divided into two on the basis of the adoral spiral of cilia and on the structure of the skeletal ring. In *Trichodina* (type *pediculus*) the adoral spiral makes one complete turn of the body and myonemes are absent. In *Vauchomia* n. g. (type *nephritica*) the adoral spiral makes 2 to 3 turns of the body, and a well developed system of myonemes is present, associated with the skeletal ring and the posterior girdle of cilia.

BIBLIOGRAPHY

FULTON, J. F. 1923 *Trichodina pediculus* and a new closely related species. Proc. Boston Soc. Nat. Hist. **37**: 1–29.

IBARA, Y. 1931 A new species of Trichodina from a salamander. J. Elisha Mitchell Scient. Soc. **46**: 214–217.

MUELLER, J. F. 1932 *Trichodina renicola,* a ciliate parasite of the urinary tract of *Esox niger.* Roosevelt Wildlife Ann. **3**: 139–154.

————1937 Some species of *Trichodina* from freshwater fishes. Tr. Am. Micr. Soc. **56**: 177–184.

EXPLANATION OF PLATE, p. 258

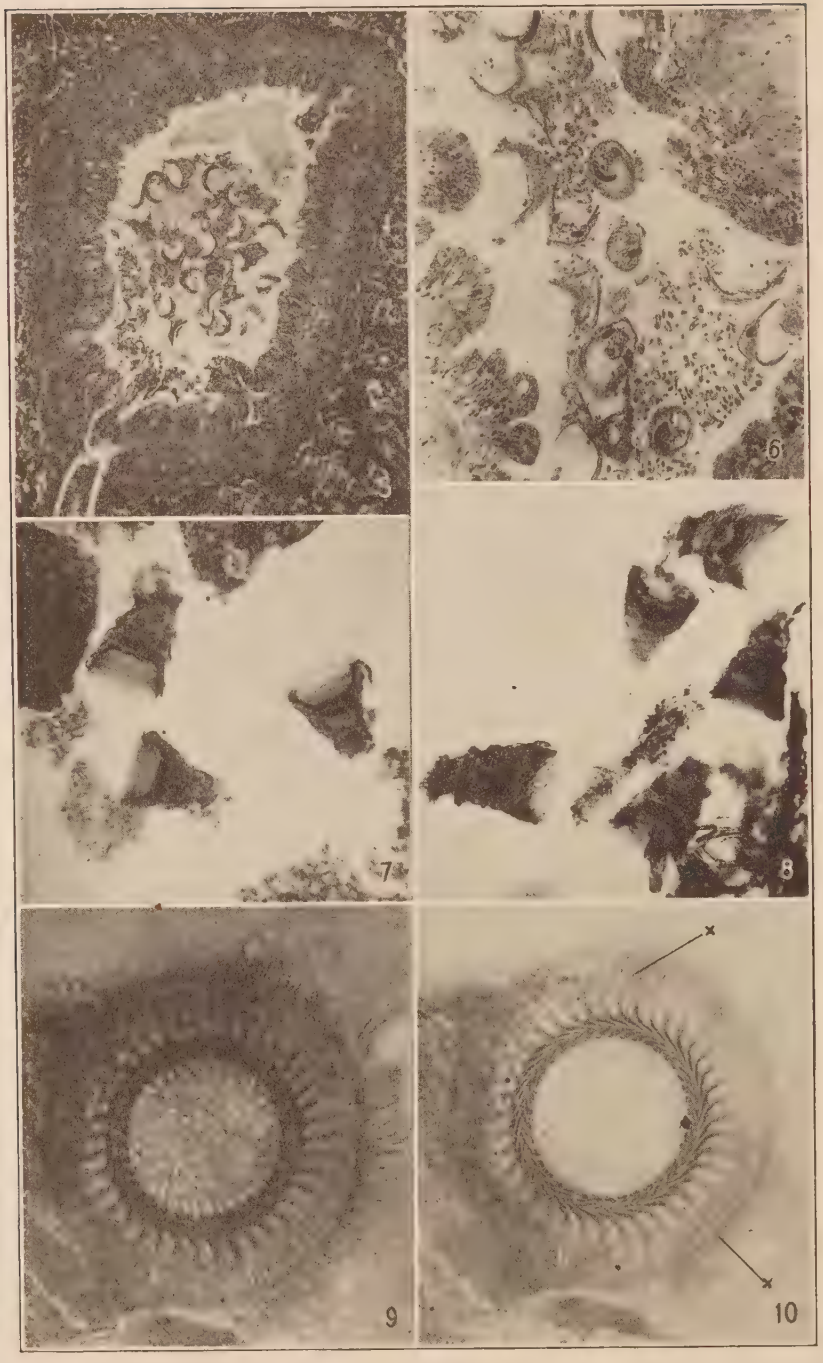
Fig. 5. Photomicrograph of cross section of kidney of muskalonge showing numerous ciliates in the lumen of the ureter. ×58.

Fig. 6. Cross section of a fold of the urinary bladder of the muskalonge showing numerous ciliates and epithelial debris in the cavity. ×160.

Figs. 7, 8. Stained whole mounts of *Vauchomia nephritica.* Adoral spiral shows clearly in lower left individual in Fig. 8. ×160.

Fig. 9. Skeletal ring, focussed at level of striated ring. ×640.

Fig. 10. Skeletal ring, with focus at level of denticulate ring in center and myonemes at edge. Irregular rays at x, x are the soft rays or myonemes. ×640.



Vauchomia nephritica

STUDIES ON THE LIFE CYCLE OF *MARITREMA MEDIUM* (TREMATODA) AND A REDESCRIPTION OF THE SPECIES*

A. J. Sheldon

Department of Bacteriology, School of Medicine, University of Oklahoma

Maritrema medium (Van Cleave and Mueller, 1932) was first described as *Microphallus medius* from the intestine of *Perca flavescens*. Mueller (1934) removed it to the genus *Maritrema,* since what at first was mistaken for a seminal vesicle was later recognized to be a large cirrus sac. Van Cleave and Mueller (1934) reported the finding of this parasite in an additional host, *Ambloplites rupestris*. Since the specimens recovered were all minute, immature forms, either enclosed in a tough cyst or recently excysted, these authors stated: "Doubtless this species reaches maturity in some other host and its presence in the intestine of fishes is to be regarded as an accident. It probably lies encysted in crayfishes, or some other small aquatic animal which is eaten both by fish and the true definite host—probably a fish-eating mammal or bird." The present paper confirms their suggestion that the crayfish serves as the second intermediate host of *Maritrema medium* and experimentally establishes this species as a parasite of mammals. The metacercaria is described and figured. In addition, since the original species description was made from an immature form, a redescription from the mature adult worms is included.

Crayfishes of the species *Cambarus virilis* and *C. propiquus* were collected from the numerous lakes in the vicinity of the University of Michigan Biological Station on Douglas Lake, Michigan, during the months of June, July, and August, 1937. Metacercariae of *Maritrema medium* were recovered in varying numbers from a high percentage of the crayfishes from Douglas Lake, Mullett Lake, Munro Lake, and Black Lake. Only a single metacercaria was obtained from 30 crayfishes collected from Burt Lake; no specimens were recovered from 6 crayfishes from Carp Lake. Metacercariae of a closely related species, *Maritrema obstipum,* were present in an early collection of crayfishes from Mullett Lake, but were absent in later collections.

In every crayfish examined the cysts were found only in the main shafts of the gills (Fig. 3), no other organ of the body being parasitized. This is interesting in view of the fact that in a number of crayfishes from

Received for publication, November 12, 1937.

* A contribution from the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, Johns Hopkins University. The author is indebted to Drs. W. W. Cort and D. B. McMullen for many helpful suggestions.

which metacercariae of *Microphallus ovatus,* a closely related form were also recovered, the cysts were never found in the gills, but in the digestive glands or liver, as reported by Osborn (1919). Localization in the second intermediate host, then, perhaps constitutes another criterion for the differentiation of the genera *Microphallus* and *Maritrema.*

Metacercariae (Fig. 2) are enclosed in compact, ovoid cysts, 0.30 – 0.38 mm long, and 0.21 – 0.29 mm maximum width. Cyst (Fig. 4) has three walls: an outer thin membrane, middle thick wall measuring 0.01 mm, and inner delicate membrane lying in intimate contact with enclosed worm. Cyst walls are transparent, and internal structures easily seen. Specimens freed by rupture of cysts are essentially identical with the immature form described and figured by Van Cleave and Mueller (1932), except that the excretory bladders of encysted worms contain large numbers of dark concretions. These were soon lost after excystment.

In many of the crayfishes immature cysts were found containing metacercariae in various stages of development. In an early stage internal organization could not be made out except for the presence of large cells arranged in Y-formation, probably representing the beginning of the excretory bladder. In later stages, although the oral and ventral suckers, the digestive system, and the genital organs were well developed, the body was full of large vacuoles, and the vitellaria were absent. In mature cysts the vacuoles were absent and the vitellaria present. The developmental stages in this form possibly correspond to those described by Okabe (1937) for the metacercariae of *Loxogenes liberum* Seno.

In view of the early report of *Maritrema medium* from fish by Van Cleave and Mueller (1932) and their later suggestion (Van Cleave and Mueller, 1934) that this is probably a parasite of fish-eating mammals or birds, mature cysts were removed from crayfishes and fed to tadpoles, perch, catfishes, mice, man, a tern, a herring gull, and canaries. Viable cysts and a few excysted forms were recovered from the intestine of the tadpoles and perch 48 hours after feeding. The excysted forms showed no development beyond that reached in the mature cyst. Further development took place only in mice. Following the feeding of approximately 200 mature cysts to 13 mice, adults were recovered from the intestines of 5 mice on the 2nd, 4th, 5th, 8th, and 9th days, respectively. The number of worms recovered was small, ranging from 1 to 5. The adults developed rapidly, fully mature worms being present on the 8th day. The worms recovered on the 2nd, 4th, and 5th days after feeding contained only few eggs and were not, therefore, mature. The fully mature adults are veritable egg sacs.

Mature adults of *Maritrema medium* from experimentally infected mice were morphologically identical with the immature form described

from fish by Van Cleave and Mueller (1932), except for size relationships, presence of uterus, and distribution of vitellaria. Because of these differences, a specimen is figured here (Fig. 1).

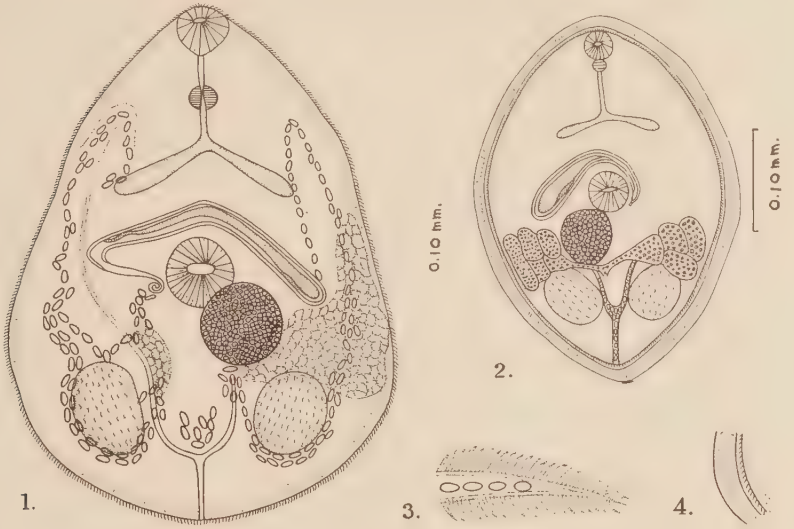


Fig. 1. *Maritrema medium,* adult from mouse. Dorsal view.
Fig. 2. Metacercaria of *M. medium* from crayfish. Ventral view.
Fig. 3. Diagram showing the position of the metacercariae of *M. medium* in the gill shaft of crayfish, enlarged.
Fig. 4. Diagram showing the cyst wall of the metacercaria of *M. medium* from crayfish, enlarged.

In flattened, fixed specimens, body averages 0.51 by 0.33 mm, oral sucker 0.05 mm, acetabulum 0.07 mm, ovary 0.08 mm, and testes 0.08 mm. Cirrus 0.20 mm. Esophagus approximately 0.10 mm long. Pharynx located about midpoint between oral sucker and branching of intestinal ceca, averaging 0.03 mm in diameter. Intestinal ceca, approximately 0.10 mm long, terminate some distance anterior to acetabulum. Eggs average .02 by .01 mm. Uterus confined mostly to posterior part of body with lateral loops which extend anteriorly on either side almost to level of pharynx. Genital pore opening at left border of acetabulum. Cirrus highly developed, extending anteriorly and to right of acetabulum. Testes lateral; ovary dextral and anterior to right testis. Vitellaria composed of scattered, loose, small follicles, not of small numbers of large follicles lying in compact masses posterior to acetabulum, as in metacercariae and immature adults. These have almost disappeared from left side of body and extend beyond anterior margin of acetabulum on right. In older forms in which many more eggs were present than in specimen of Fig. 1, vitellaria had almost disappeared from both sides of body, with only small follicles present posterior to acetabulum. It seems probable that greatest portion of vitellaria had been used up in production of eggs. Excretory bladder Y-shaped.

The negative results obtained from the above feeding experiments with fishes and birds, together with the fact that no natural infections with *Maritrema medium* were found in a number of fishes and birds

examined from localities in which crayfishes had been found to be heavily infected with metacercariae, would seem to rule out these animals as hosts of the adult of this parasite. Fishes, however, may serve as transfer hosts. Although mice could be readily infected experimentally, only few worms developed following the feeding of large numbers of mature cysts. Apparently, the laboratory mouse is not a very favorable host for *M. medium*.

Bibliography

Mueller, J. F. 1934 Note on *Microphallus obstipus* and *M. medius* Van Cleave and Mueller. Proc. Helm. Soc. Wash. **1**: 5.

Okabe, Koyo. 1937 On the life history of a frog trematode, *Loxogenes liberum* Seno. Annat. Zool. Jap. **16**: 42–52.

Osborn, H. L. 1919 Observations on *Microphallus ovatus* sp. nov. from the crayfish and black bass of Lake Chautauqua, New York. J. Parasitol. **5**: 123–127.

Van Cleave, H. J., and J. F. Mueller. 1932 Parasites of Oneida Lake Fishes. Part 1. Descriptions of new genera and new species. Roosevelt Wildlife Ann. **3**: 1–71.

————1934 Parasites of Oneida Lake Fishes. A biological and ecological survey of the worm parasites. Roosevelt Wildlife Ann. **3**: 161–334.

A NEW STRIGEID CERCARIA WHICH PRODUCES A BLOAT DISEASE OF TADPOLES[1]

W. W. Cort and Sterling Brackett

In the summer of 1932 a new strigeid cercaria was found in one specimen each of *Stagnicola palustris elodes* (Say) and *Stagnicola emarginata angulata* (Sowerby) from two locations on the shore of Douglas Lake, Michigan. This cercaria penetrated actively in tadpoles and developed into a diplostomulum. Heavily infected tadpoles became very much bloated and died from the effects of the parasites. The two snail infections were evidently old since they died out before it was possible to complete the study of the structure of the cercaria. This species of cercaria did not turn up again in the collections until the summers of 1936 and 1937 when it was found in *S. exilis* (Lea) from Sedge Pool, a small beach pool on the north shore of Douglas Lake. One positive was found in 1936 and two in 1937 out of several hundred specimens of this species of snail examined from this pool.

From this material it was possible to complete the study of the structure of the cercaria, repeat the infection experiments on tadpoles, and describe the fully developed diplostomulum. Attempts were also made to find the adult by experimentally feeding the fully developed metacercariae to several different species of birds and mammals. So far all the experiments have been negative. Since this form is so rare in the Douglas Lake region that the chances seem against the immediate working out of its complete life cycle, we have decided to publish the work so far completed.

The methods used in the study of the anatomy and activity of the cercaria were exactly the same as those described in detail in a recent paper (Cort and Brackett, 1937). Most of the observations on the diplostomulum were made from unstained living specimens.

On account of the relation of this species of cercaria to frog tadpoles we propose for it the name *Cercaria ranae* n. sp.

Cercaria ranae n. sp.

(Fig. 1)

Specific diagnosis: Small strigeid cercaria, with body and furcae both shorter than tail stem; body length[2] 119–203 μ, mean 163 ± 1.8 μ; distance from middle of

Received for publication, November 18, 1937.

[1] A contribution from the University of Michigan Biological Station and the Department of Helminthology, School of Hygiene and Public Health, the Johns Hopkins University.

[2] Unless otherwise indicated the measurements are of 50 specimens. The probable error of the mean and the extremes are used to indicate the character of the variation.

ventral sucker to posterior end of body 49–70 μ, mean 58 ± 0.5 μ; body width 35–50 μ, mean 41 ± 0.5 μ; tail stem length 168–286 μ, mean 246 ± 1.9 μ; tail stem width 28–42 μ, mean 38 ± 0.5 μ; furcal length 154–236 μ, mean 210 ± 1.4 μ. Oral sucker (25 measurements), length 30–41 μ, mean 34 ± 0.4 μ; width 26–33 μ, mean 28 ± 0.3 μ. Ventral sucker diameter (25 measurements) 20–33 μ, mean 22 ± 0.2 μ. Prepharynx very short; pharynx large with diameter (24 measurements) 12–17 μ, mean 13 ± 0.2; esophagus bifurcates about half way back to ventral sucker; well developed intestinal ceca, constricted into series of sacculations, extend almost one-half distance from posterior margin of ventral sucker to posterior end. Two "unpigmented eye-spots" near lateral margins of body in front of penetration glands. No forward-pointing spines at anterior tip in circumoral spineless area; spines of oral cap extend about half length of oral sucker; rest of body, tail stem, and furcae covered with small sparsely scattered spines; single circle of very long spines on ventral sucker. Penetration glands four in number in front and to sides of ventral sucker and ventrad of intestinal ceca, with pair of ducts running forward on each side and expanding considerably after entering oral sucker. Genital primordium a mass of cells in front of bladder. No caudal bodies in tail stem. Excretory system with large bladder, postacetabular commissure and seven flame cells on each side in body and three in tail; "island of Cort" large and branches of bladder in tail stem as in other strigeid cercariae.

From *Stagnicola emarginata angulata* (Sowerby), *S. palustris elodes* (Say), and *S. exilis* (Lea) in Douglas Lake region, Michigan, U. S. A.

Daughter sporocysts thin-walled with series of sacs packed with cercariae separated by constrictions; anterior tip (fig. 2) of sporocyst narrow and very mobile; area around birth pore only slightly protruding.

The cercariae of this species appear to emerge from the snail host continuously but more abundantly during the day. When the water is undisturbed they hang motionless with body downward and furcae forming an angle of about 90° with each other. They tend to sink slowly. From time to time individual cercariae will swim upward for varying distances. The periods of rest are much longer than those of swimming, so that only about 5 per cent of the cercariae appear to be in motion at any one time. Swimming may be in any direction and the cercariae often change their direction once or even several times during one period of swimming. They, therefore, may come to rest at any angle, although as they sink they tend to assume a perpendicular position. Apparently there is little if any reaction to light.

Certain points in the structure of *C. ranae* merit further mention. The spines around the ventral sucker are quite different from those of most other strigeid cercariae since they are in one row and very long. It is also unusual to find both the stem and furcae of the tail covered with spines. The pharynx is large and the intestinal ceca stand out prominently back of the ventral sucker. While the penetration glands in greatly extended specimens will be entirely in front of the ventral sucker, in more contracted ones they appear both in front and to the sides as shown in fig. 1.

The excretory system (fig. 1) was worked out in detail and is so characteristic that a further description seems worth while. The bladder when fully distended is very large and gives the appearance of being

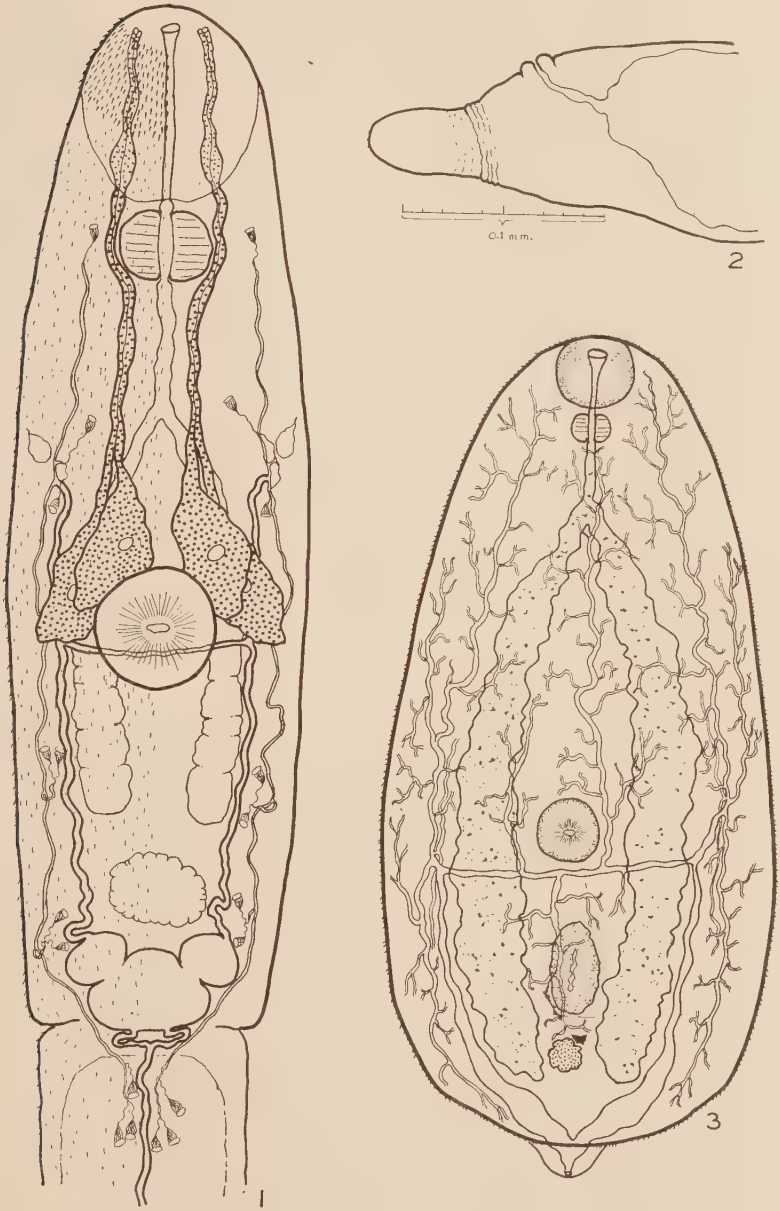


Fig. 1.—Semidiagrammatic drawing of the body of *C. ranae* showing structural details, ventral view.

Fig. 2.—Camera lucida drawing of the anterior end of a daughter sporocyst of *C. ranae*.

Fig. 3.—Composite sketch of the diplostomulum of *C. ranae*, ventral view.

three-lobed, on account of the expansion of the beginnings of its arms into saccular structures. The "island of Cort" is unusually large and the caudal branch from the bladder extends back from it down the tail stem to bifurcate and open on the dorsal sides of the furcae at about the middle of their length. A commissure just back of the ventral sucker connects the anterior branches of the bladder, which extend forward from it to a point on each side just back of the so-called "unpigmented eyespots" where they connect with the anterior and posterior collecting tubes. One flame cell at the level of the pharynx and one pair near the "unpigmented eyespots" connect with each of the anterior collecting tubes. Two pairs of flame cells on each side in the body connect with the posterior collecting tubes; and there are three flame cells on each side close to the base of the tail on the extension of the posterior collecting tubes into the tail stem.

The daughter sporocysts are only found imbedded in the digestive gland with their anterior ends projecting out slightly from its surface. They are very fragile and thin-walled and it was impossible to free whole sporocysts for study. The longest fragment seen measured about 3 mm. A number of constrictions divide each sporocyst into a series of sacs which are packed with cercariae in various stages of development. The anterior ends of the sporocysts (fig. 2) are rather narrow and are very mobile and protrusible. The birth pore is surrounded by a slightly protruded area and the birth canal is rather short.

COMPARISON WITH OTHER STRIGEID CERCARIAE

Cercaria ranae exhibits the following combination of rather conspicuous structural characters which make it easy to differentiate from other strigeid cercariae: four penetration glands in front of the ventral sucker, absence of caudal bodies in the tail stem; a commissure connecting the arms of the excretory bladder behind the ventral sucker; and two groups of three flame cells in the tail stem close to the body. In our review of the literature we have found descriptions of 13 different species of strigeid cercariae in which there are four penetration glands in front of the ventral sucker. Four of these species, *Cercaria tenuis* Miller, 1923, *C. bajkovi* McLeod, 1934, *C. indicae* XXII Sewell, 1922, and *C. letifera* Fuhrmann (Dubois, 1929), differ strikingly from *C. ranae* in having definite caudal bodies in the tail stem and in the absence of any bladder commissure. For the other nine no caudal bodies are described in the tail stem. Three of these, *C. marcianae* Cort and Brooks, 1928, *C. indicae* I Sewell, 1922, and *C. tropicalis* Faust and Hoffman, 1934, lack the bladder commissure and have four flame cells in the tail stem.

Another group of these cercariae, which includes the cercariae of *Cotylurus cornutus* and *C. flabelliformis* besides *C. sanjuanensis* Miller,

1927, and *C. fissicauda* La Valette (Brown, 1926), have the bladder commissure in front of the ventral sucker and four flame cells in the proximal part of the tail. Finally the only two of these species that have the bladder commissure back of the ventral sucker, *C. helvetica* XIV and *C. helvetica* XXIX described by Dubois (1929), have four flame cells in the proximal part of the tail. For these two cercariae no statement is made in regard to caudal bodies. Both, however, differ from *C. ranae* in having forward pointing spines at the anterior tip, three rows of spines around the ventral sucker, and a larger number of flame cells in the body.

The comparison of the series of strigeid cercariae just given indicates that resemblance in certain larval characters in the cercariae of this group does not necessarily mean systematic relationship. For example *C. ranae,* which develops into a diplostomulum in tadpoles, resembles in the number and position of the penetration glands and in the absence of caudal bodies the cercaria of *Cotylurus flabelliformis* and *C. marcianae.* The first of these develops into a tetracotyle in snails and the second into a mesocercaria, similar to that of *Alaria mustelae,* in tadpoles. It is apparent, therefore, that the three species cannot be very closely related. In fact in the present state of our knowledge it is not possible to assign strigeid cercariae to subdivisions of the STRIGEOIDEA, from their structural characters alone.

Diplostomulum of C. ranae (fig. 3)

C. ranae penetrates into frog tadpoles and develops into a very characteristic diplostomulum (fig. 3). The fore-body of this diplostomulum, which is about twice as long as wide, is flattened, ovate, and slightly concave ventrally, especially toward the posterior end. The hind-body is represented by a small conical projection at the posterior end with the excretory pore at its tip. The entire surface of the body is covered with very small spines. The ventral sucker, which is distinctly back of the middle of the body, is slightly smaller than the oral. The holdfast organ is rather small and inconspicuous and has a longitudinal slit-like external opening. All that could be made out of the genital primordium is shown in the drawing as a small irregular body just back of the holdfast organ. No traces of lateral suckers are present. The digestive system has a rather small subventral mouth with the buccal cavity surrounded by a rather large oral sucker. The prepharynx is very short and the well developed pharynx is distinctly broader than long. The short narrow esophagus is followed by very large intestinal ceca with irregular outlines, which have a very characteristic yellowish tinge in living specimens from the contained food material. The branches of the primary excretory system and the flame cell pattern were not worked out, except the anterior and posterior collecting tubes which open into the anterior branches of the

bladder a short distance in front of the commissure. The arrangement of the excretory system of the diplostomulum of *C. ranae,* as shown in fig. 3, is quite characteristic in the position of the cross commissure of the bladder back of the ventral sucker and in the absence of any anterior commissure connecting the lateral and median anterior branches of the reserve bladder. The branches of the reserve bladder contain the usual concretions which are not shown in the figure.

Measurements of 50 specimens of the diplostomulum of *C. ranae* from a 45-day-old infection killed in 5 per cent hot formalin gave the following dimensions: length 336–686 μ, mean 456 ± 8.0 μ;[3] width 210–336 μ, mean 265 ± 2.8 μ. Since it was impossible in the formalin-killed material to measure the suckers and other smaller structures another series of measurements was made of 50 specimens selected at random from an 18-day-old infection. These specimens had been killed in hot Gilson's fixative, stained with Ehrlich's hematoxylin and mounted in balsam. This series of measurements is shown in table 1. The much smaller size of the diplostomula of this series as compared with those 45 days old, indicates an increase in size with age after the metamorphosis from the cercaria body is completed. We, therefore, made a further comparison of diplostomula

TABLE 1.—*Measurements in micra of 50 specimens of the diplostomulum of C. ranae from an 18-day-old infection. From toto mounts of specimens killed in hot Gilson's fixative, stained with Ehrlich's hematoxylin and mounted in balsam.*

	Minimum	Maximum	Mean with probable error of mean
Body length	280	490	366 ± 3.7
Body width	140	224	178 ± 1.5
Anterior end to middle of ventral sucker	168	280	201 ± 2.5
Width of oral sucker	33	46	39 ± 0.2
Width of pharynx	13	20	17 ± 0.2
Width of ventral sucker	33	43	38 ± 0.3
Length of holdfast organ	33	56	43 ± 0.5
Width of holdfast organ	20	36	27 ± 0.4
Length of esophagus	10	40	21 ± 0.6

from 15-day and 45-day-old infections. Both series were killed in hot 5 per cent formalin and measured without mounting. Measurements of 10 specimens of the 15-day-old series gave the following averages: length 351 μ; width 178 μ; oral sucker 40 μ; ventral sucker 35 μ. The measurements of 10 specimens of the 45-day-old series gave the following averages: length 531 μ; width 256 μ; oral sucker 52 μ; ventral sucker 50 μ. Whether the diplostomula had completed their growth at 45 days is not known, because specimens from older infections were not available for measurement. These data indicate that too much weight cannot be given to measurements of diplostomula as specific characters, especially when

[3] The extremes and the probable error of the mean are used to give the character of the variations.

specimens from natural infections, the age of which would be unknown, are used.

It would be rather futile to try to compare the diplostomulum of *C. ranae* with all described strigeid metacercariae of this type since such a large proportion of them are insufficiently described. It is easily distinguished from the other diplostomula we have seen in fish and tadpoles at the Biological Station in the following characteristics: the large size and yellowish color of the intestinal ceca; the position of the cross commissure of the excretory bladder back of the ventral sucker; the absence of any anterior commissure connecting the lateral and median anterior branches of the reserve bladder; and the absence of any trace of lateral suckers on the anterior end. In fact this combination of characters sets the diplostomulum of *C. ranae* off as distinct from all the others of which we have found adequate descriptions in the literature.

DEVELOPMENT IN TADPOLES

Under the conditions of warm summer weather the development of the diplostomula of *C. ranae* is quite rapid in the experimentally infected tadpoles. No attempt was made to follow in detail the changes during the metamorphosis from the cercaria body. Forty hours after the penetration of the cercariae, the penetration glands had disappeared, the shape of the body had changed, and the intestinal ceca had become very prominent. In one experiment some diplostomula that had completed their metamorphosis were found in a tadpole that had been first exposed to infection 10 days previously, in spite of the fact that the infection was extremely heavy. In a tadpole with a lighter infection, nothing but completely metamorphosed diplostomula were found 16 days after the last exposure.

The diplostomula in the tadpole are found in the body cavity and widely distributed in the tissues. No evidence could be seen of the development of cysts around them either of parasite or host origin. Very heavy infections in the tadpoles caused rather severe symptoms, producing a condition to which we gave the name "bloat disease" on account of the characteristic distension of the abdomen. In severe cases the abdomens of the infected tadpoles become greatly distended with gas. Soon they become very sluggish, lose equilibrium, and will be found floating on their backs (fig. 4). Death usually occurs within a few days after these symptoms. Closer examination of these tadpoles shows accumulations of gas in the body cavity and in the tissues surrounding the clumps of the metacercariae. Numerous small hemorrhagic areas are also present. These extreme symptoms leading to the death of the tadpoles occur only in individuals given extremely heavy infections and begin to show up about 8 to 10 days afters the cercariae have penetrated. Most of the experi-

FIG. 4.—Photograph of a tadpole with "bloat disease" from an extremely heavy infection with *C. ranae*.

mentally infected tadpoles continue to live for a month or more, and even those with moderately heavy infections showed only slight indications of bloating. On account of the extremely heavy infection necessary to produce "bloat disease" severe enough to produce the death of the tadpoles, it seems probable that death from this cause would rarely if ever occur naturally. In a number of cases dead bloated tadpoles have been picked up but in none of them have diplostomula of this species been found. It was concluded, therefore, that in these cases the bloating had occurred after death. It is of interest in this connection to note that Rankin and Hughes (1937) stated that salamanders heavily infected with *Diplostomulum ambystomae* were somewhat bloated and sluggish.

SUMMARY

A new species of strigeid cercaria, *C. ranae* n. sp., is described from *Stagnicola palustris elodes, S. emarginata angulata,* and *S. exilis* from the Douglas Lake region in the northern part of the southern peninsula of Michigan. This species is distinguished from other strigeid cercariae, which also have four penetration glands in front of the ventral sucker, by the following combination of prominent characteristics: absence of caudal bodies; presence of a bladder commissure back of the ventral sucker; absence of forward pointing spines at the anterior tip; presence of a single row of very long spines around the ventral sucker; and the presence of two groups of three flame cells in the proximal part of the tail. The daughter

sporocysts are divided by constrictions into a series of sacs packed with cercariae. Their anterior ends have a narrow, very mobile and protrusible tip. The birth pore is surrounded by a slightly protruded area and the birth canal is rather short.

C. ranae penetrates into frog tadpoles and metamorphoses in 10 to 16 days into a diplostomulum, with the following rather striking combination of characters: absence of lateral suckers; large size and yellow color of the intestinal ceca; presence of a cross commissure of the excretory bladder back of the ventral sucker; and absence of any anterior commissure connecting the lateral and median branches of the reserve bladder. These diplostomula continue to grow after the metamorphosis from the cercaria body is completed up to at least 45 days after infection. They are found in the body cavity and widely distributed in the tissues of the tadpole. In very heavy infections they cause a characteristic "bloat disease" in the tadpoles. Experimental attempts to obtain the adult of this species have, up to the present time, been negative.

Bibliography

Brown, F. J. 1926 Some British freshwater larval trematodes with contributions to their life histories. Parasitology **18**: 21–34.

Cort, W. W. and Sterling Brackett. 1937 Two new species of strigeid cercariae from the Douglas Lake region, Michigan. J. Parasitol. **23**: 265–280.

Cort, W. W. and S. T. Brooks. 1928 Studies on holostome cercariae from Douglas Lake, Michigan. Tr. Am. Micr. Soc. **47**: 179–221.

Dubois, G. 1929 Les cercaires de la region Neuchatel. Extrait Bull. Soc. Neuchateloise Sc. Nat. **53**(n.s. **12**) : 1–177.

Faust, E. C. and W. A. Hoffman. 1934 Studies on *Schistosomiasis mansoni* in Puerto Rico. III. Biological studies. 1. The extra-mammalian phases of the life cycle. Puerto Rico J. Pub. Health and Trop. Med. **10**: 1–97.

McLeod, J. A. 1934 Notes on cercarial dermatitis with descriptions of the causative organisms, *Cercaria wardlei* n. sp., *Cercaria bajkovi* n. sp., and the parthenogenic stage of *Cercaria elvae* Miller. Canad. J. Res. **10**: 394–403.

Miller, H. M., Jr. 1923 Notes on some furcocercous larval trematodes. J. Parasitol. **10**: 35–46.

————1927 Furcocercous larval trematodes from San Juan Island, Washington. Parasitology **19**: 61–83.

Rankin, J. S., Jr. and R. C. Hughes. 1937 Notes on *Diplostomulum ambystomae* n. sp. Tr. Am. Micr. Soc. **54**: 61–66.

Sewell, R. B. S. 1922 Cercariae indicae. Indian J. Med. Res. **10**(Supplementary number) : 1–370.

OBSERVATIONS ON PRECOCIOUS METACERCARIAL DEVELOPMENT IN THE TREMATODE SUPER-FAMILY PLAGIORCHIOIDEA*

Donald B. McMullen
Department of Biology, Monmouth College

INTRODUCTION

Three general types of developmental anomalies in larval trematodes have been described in the literature. One of these is the appearance of monsters, especially of the "Siamese twin" type (Mathias, 1930). Another is the development of extra flame cells and variations in pigment distribution (Rothschild, 1936). The third type is the precocious or *progenetique* development of the larval stages. The latter has been frequently observed, especially in the sexual maturity attained by the metacercariae of certain species of trematodes within the second intermediate host.

The precocious development of the metacercariae within the sporocyst has been previously reported at least six times. It is evident that it is not at all uncommon. Cort (1915) and McMullen (1937b) found it in infections of *Plagiorchis proximus;* Tanabe (1922) and McMullen (1937a, b) found it in *Plagiorchis muris;* Cort and Brackett (1937) report it in *Diplostomum flexicaudum;* and Wesenberg-Lund (1934) states that the condition is common in snails infected with xiphidiocercariae. The latter author gives some evidence and expresses opinions regarding the causes of these anomalies as he found them in the snails collected in Denmark. He found that the metarcercariae were more common in snails kept in the laboratory for long periods than in those taken directly from natural habitats. He also reports getting encystment in an excised digestive gland kept at 22°. His conclusions are that unfavorable living conditions, *i.e.,* poor food, low temperatures, old age and death, are responsible for the precocious development. Cort and Brackett found that the precocious development in *Diplostomum flexicaudum* took place in the older infections. In general the observations considered here substantiate some of these conclusions.

During the summers of 1935–37 Dr. W. W. Cort, Mr. Sterling Brackett and the author made extensive collections of *Stagnicola emarginata angulata* (Sowerby) and *Heliosoma campanulatum smithii* (Baker) in the region of the University of Michigan Biological Station, and

Received for publication, December 19, 1937.
* A contribution from the University of Michigan Biological Station. The author is indebted to Dr. W. W. Cort and Sterling Brackett for valuable assistance on this problem.

examined them for infections of larval trematodes. The 11,105 specimens of *Stagnicola,* were collected at intervals from North Fish-tail Bay and Phragmites Flats on Douglas Lake and from the beach east of the mouth of Maple River on Burt Lake. (For exact location of the collecting areas see Cort, McMullen and Brackett, 1937, Map I, p. 506.) The *Heliosoma,* 1,229 specimens, were collected from Twin Lakes and the small neighboring Bullfrog Lake. The digestive gland of each snail was torn apart in physiological salt solution and examined under a microscope. Particular attention was paid to the identification of the larval trematodes and the incidence of multiple infections (Cort, McMullen and Brackett, 1937). In the summer of 1937 an attempt was made to determine the age of the infections.

A relatively large percentage of the snails examined was infected with xiphidiocercariae so it was possible to observe great numbers of these larval trematodes to detect any anomalies. None of the first two types mentioned above were found. In the normal life cycle of trematodes having xiphidiocercariae the cercariae emerge from the snail, penetrate and encyst in the second intermediate host. In six of the species of xiphidiocercariae we found the development of normal and abnormal metacercariae within the sporocyst, *i.e.,* precocious development.

The terms normal and abnormal metacercariae, as applied to these precocious forms, are used because some of the cercariae were able to develop into normal, infective metacercariae and others were unsuccessful. The normal metacercariae of this group of trematodes have a cyst wall, concretions fill the excretory bladder, the stylet is usually loose in the cyst cavity, and the tail has been lost. Many of the precocious forms seemed to be normal metacercariae. Variations in the development were not uncommon and included: cercariae with partial deposition of granules in the bladder, with the tail present but degenerate, and with the stylet still in position; others had a greater deposit of concretions in the bladder, the stylet was absent and the tail still more degenerate; most striking of all were those which had increased greatly in size, and had adult organs well developed but still retained a stunted tail.

The present paper presents additional notes on the precocious development in *Plagiorchis muris* Tanabe and *P. proximus* Barker, and new observations of the phenomenon in *Alloglossidium corti* (Lamont), *Macroderoides typicus* (Winfield), *Plagiorchis micracanthos* Macy and *Cercorchis medius* (Stunkard).

OBSERVATIONS

Macroderoides typicus. It has been demonstrated experimentally that the cercariae of *M. typicus* will penetrate and develop into normal metacercariae in tadpoles, salamander larvae and bullheads. Snails were

exposed to the cercariae and penetration and encystment were observed but the metacercariae died in a short time (McMullen, 1935).

In the collections made during the summer of 1937 there were 376 snails, *Heliosoma campanulatum smithii,* infected with *M. typicus.* Out of this number 3, or about 0.8 percent, had a few abnormal metacercariae. In these metacercariae (fig. 1) the body was considerably larger than in the cercaria, the stylet was usually absent and the tail was attached but practically useless. The intestinal ceca were large and filled with large, opaque particles. The testes, ovary and anlage of the cirrus could be seen. The bladder was very much enlarged and contained clumps of small concretions. Except for the presence of the tail these resemble the older excysted, infective metacercariae of *M. typicus.*

Alloglossidium corti. In this species the cercariae emerge from the first intermediate host, penetrate into a variety of arthropods and encyst (McCoy, 1928; McMullen, 1935; Crawford, 1937). In the 380 snails, *Heliosoma campanulatum smithii,* infected with *Alloglossidium corti* 26 or nearly 7 percent, showed precocious development. In a few cases normal, encysted metacercariae (fig. 2), measuring 0.13×0.10 mm, were found. In others the metacercariae did not develop normally and were not encysted. Three of these precocious, abnormal metacercariae were seen in one sporocyst (fig. 3). The sporocyst wall was not pigmented but in certain areas, between the metacercariae, there were orange-colored, noncellular masses. The bodies of the metacercariae were about twice the size of those of the cercariae, the stylets were absent and the excretory bladders were filled with a transparent fluid. In some the tail was still attached. In one snail there were larger unencysted forms, about three times the size of the cercaria (fig. 4). In these the adult organs had developed to a point where they could almost begin egg production, a stage reached in the definitive host in 30–40 days.

Plagiorchis proximus. The cercariae of *P. proximus* emerge from the snail host and it has been demonstrated experimentally that they will penetrate and produce normal metacercariae in several species of arthropods. Metacercariae of this species have been previously reported from snails (Cort, 1915; McMullen, 1937b) but the exposure of snails to cercariae failed to give infections.

In 324 snails, *Stagnicola emarginata angulata,* having infections of *Plagiorchis proximus* there were 19, or nearly 6 percent, with precocious development. In some of these infections a tendency toward abnormal development was shown by the stunted, useless tail and the beginning of the deposit of concretions in the excretory bladder (fig. 5). These larval trematodes were sluggish, some were larger and others smaller than the normal cercaria. The stylet was often gone and the stylet glands had disintegrated. In more advanced stages of this development (fig. 6)

the body was larger, the tail more degenerate and the excretory bladder was packed with concretions. In a few cases cysts, measuring 0.12 mm in diameter, containing normal metacercariae (fig. 7) were found.

Plagiorchis micracanthos. The cercariae of *P. micracanthos* normally emerge, penetrate into various insect larvae and encyst (McMullen, 1937b). In our collections 19 *Stagnicola emarginata angulata* were found to be infected with *P. micracanthos* and one of these had a few cysts containing normal metacercariae. These were practically indistinguishable from the metacercariae of *Plagiorchis proximus* (fig. 7).

Plagiorchis muris. Normally the cercariae of *P. muris* emerge from the snail host, penetrate into various insect larvae and encyst (Tanabe, 1922; McMullen 1937b). Precocious metacercariae in the snails were reported by both authors. The precocious nature of these metacercariae was demonstrated by the fact that it was impossible to get penetration and encystment of the cercariae in experimental snails. It has also been demonstrated that these precocious metacercariae are infective.

In the 905 *Stagnicola emarginata angulata* infected with *Plagiorchis muris* there were 90, or nearly 10 percent, with precocious development. The precocious metacercariae seen in these infections can be divided into three general types. In a great many snails there were numbers of sporocysts containing 5–9 fully developed, encysted metacercariae (fig. 8). In a few cases one could see that there were two definite age groups in the mass of encysted metacercariae from one snail. In most cases, however, they were either approximately the same age or were in a graded series. The older metacercariae were larger than any seen in the natural second intermediate hosts. The cysts measured as large as 0.24×0.32 mm and the larger they became the more elongate they were. From observations on the development of these metacercariae in the normal second intermediate host it must be concluded that it takes at least a month and probably a much longer time for these metacercariae to develop.

In several infections numbers of large excysted, immature flukes were found. In these the adult organs were well advanced, resembling young trematodes of this species taken from experimental animals (McMullen, 1937b, fig. 5). These young trematodes were evidently the oldest of the precocious forms. In a few infections of *Plagiorchis muris* there were abnormal forms that had not encysted (fig. 9). These had a degenerate tail and bodies that were larger than the cercaria. The excretory bladder was filled with concretions and the stylet and stylet glands had disappeared.

Cercorchis medius. The cercariae of *C. medius* normally emerge from the snail host and it has been demonstrated that they penetrate and encyst in tadpoles (McMullen, 1934). Precocious development in the snail host has not been reported in this species. In 9 *Heliosoma*

campanulatum smithii infected with *C. medius* one had a few normal, encysted metacercariae (fig. 10). The stylet was visible in the cyst cavity, the bladder was filled with concretions and the cysts averaged 0.14 mm in diameter.

SEASONAL VARIATION

In all the species of trematodes, where there were enough showing precocious development, there was evidence that the incidence increased as the summer progressed. In two species, *Alloglossidium corti* and *Plagiorchis muris,* there were sufficient numbers to make an analysis.

The first collection of *Heliosoma campanulatum smithii* was made June 21. Infections of *A. corti* were found in 268 snails and of these 13, or nearly 5 percent, showed precocious development. The other collection was made August 26 and of these 112 snails were infected with the same trematode. Of these 13, or nearly 12 percent, showed precocious development. This marked increase in the incidence during the summer months is of interest and may help explain the cause of the phenomenon.

With the larger number of infections showing precocious development in *P. muris,* and with periodic collections throughout the summer it was possible to get a clearer picture of the seasonal variation. In the collections June 7–14 a little over 6 percent of the infected snails had precocious larvae; June 28–July 12 more than 8 percent; July 27–August 6 nearly 12 percent; and August 20–23, 22 percent. This shows a nearly three-fold increase of precocious development in 12 weeks.

In all the cases observed, with one exception, the metacercariae were found in infections that were "middle aged" or older. In one infection of *Plagiorchis muris* well developed metacercariae were found in old sporocysts but in the same snail there were numbers of young sporocysts. It may be that this was a case of superimposed infection. At any rate none of the metacercariae were seen in the young sporocysts.

All the snails used in the calculations given above were mature specimens. In the last collections of the summer, a number of immature snails, *Stagnicola emarginata angulata* with shells averaging 9×14 mm, were brought into the laboratory. In this group of 514 snails only 5 percent, as compared with 22 percent in the mature snails, were found to have precocious forms of *P. muris.* It is of interest that the percentage of immature snails harboring these precocious metacercariae is nearly the same as that found in the collections early in the summer. It is believed that these infections are carried over the winter and that they are the ones to be found in the snails early in the following summer.

It was also noted that collections made at the same time but in different areas may show wide variations in the percent of snails harboring precocious metacercariae. In the last collections made on Burt Lake and

North Fish-tail Bay there was a difference of 8 percent. This indicates that environmental factors may play a part in causing these anomalies.

DISCUSSION

From these observations on precocious development it is possible to draw certain inferences regarding the possible causes of the phenomenon. Striking differences in the percentages in snails taken from different areas show that environmental conditions and the resulting changes in the physiological condition of the snails are factors to be considered. With the xiphidiocercariae discussed in the present paper temperature does not seem to have a marked effect. In the early summer when the water is quite cold the incidence is low. As the summer advances and the water warms the incidence increases but toward the end of the summer when the water begins to cool off again the incidence of precocious forms continues to increase. Most of the snails that were examined were mature at the beginning of the summer season. During the summer months these snails were approaching senility and death. At the same time the number of mature and old infections of xiphidiocercariae increased. It seems, therefore, that the age of the snail and its trematode infection, with the resulting physiological changes, has a direct bearing on the increase in the incidence of the precocious metacercariae. The lower incidence of these anomalies in the immature snails substantiates this idea.

The snails that were examined were not kept in the laboratory for any length of time so that the precocious development observed had taken place in the natural habitats. The advanced development of many of these trematodes indicates that it had been started several weeks and perhaps months previous to the examination. This is significant because it indicates that under natural conditions the snail harbors the infections for months, rather than a few weeks as one might suppose from the observations of infected snails in the laboratory.

SUMMARY

Precocious development of larval trematodes has been found to be relatively common in *Stagnicola emarginata angulata* and *Heliosoma campanulatum smithii* harboring infections of *Macroderoides typicus* (Winfield), *Alloglossidium corti* (Lamont), *Plagiorchis proximus* Barker, *Plagiorchis micracanthos* Macy, *Plagiorchis muris* Tanabe and *Cercorchis medius* (Stunkard).

These precocious metacercariae were found in various stages of development. Some were normal metacercariae, others did not encyst and showed the beginning of the deposition of concretions within the excretory bladder while the more advanced stages resembled young adults. The incidence of this phenomenon has been found to increase during the sum-

mer months. The condition seems to be caused by the approaching senility of the snails and the trematode infections, and possibly by other physiological and ecological factors as yet unknown.

Bibliography

Cort, W. W. 1915 Some North American larval trematodes. Illinois Biol. Monogr. **1**: 447–532.

Cort, W. W., McMullen, D. B. and Brackett, Sterling. 1937 Ecological studies on the cercariae in *Stagnicola emarginata angulata* (Sowerby) in the Douglas Lake region, Michigan. J. Parasitol. **25**: 504–532.

Cort, W. W. and Brackett, Sterling. 1937 Precocious development of the metacercaria stage of *Diplostomum flexicaudum* in the snail intermediate host. J. Parasitol. **23**: 545–546.

Crawford, Wiley W. 1937 A further contribution to the life history of *Alloglossidium corti* (Lamont), with especial reference to dragonfly naiads as second intermediate hosts. J. Parasitol. **23**: 389–399.

McCoy, O. R. 1928 Life history studies on the trematodes from Missouri. J. Parasitol. **14**: 207–228.

McMullen, Donald B. 1934 The life cycle of the turtle trematode, *Cercorchis medius*. J. Parasitol. **20**: 248–250.

———1935 The life histories and classification of two Allocreadiid-like Plagiorchids from fish, *Macroderoides typicus* (Winfield) and *Alloglossidium corti* (Lamont). J. Parasitol. **21**: 369–380.

———1937a An experimental infection of *Plagiorchis muris* in man. J. Parasitol. **23**: 113–115.

———1937b The life histories of three trematodes, parasitic in birds and mammals, belonging to the genus *Plagiorchis*. J. Parasitol. **23**: 235–243.

Mathias, Paul. 1930 Sur une cercariae monstre double du type lamboide. Ann. Parasitol. **7**: 147–150.

Rothschild, Miriam. 1936 A note on the variation of certain cercariae (Trematoda). Novit. Zool. **40**: 170–175.

Tanabe, H. 1922 A contribution to the study of the life cycle of hermaphroditic distomes. A study of a new species, *Lepoderma muris*, n. sp. Okayoma Igakkai Zasshi, pp. 47–68. (Japanese.)

Wesenberg-Lund, C. 1934 Contributions to the development of the Trematoda Digenea. Part II. The biology of the freshwater cercariae in the Danish freshwaters. Mem. Acad. Roy. Soc. Let. Dan. **5**: 1–223.

Explanation of Plate, p. 280

Fig. 1.—Abnormal metacercaria of *Macroderoides typicus*.

Fig. 2.—Normal metacercaria of *Alloglossidium corti*.

Fig. 3.—Abnormal metacercariae of *Alloglossidium corti* within the sporocyst.

Fig. 4.—Abnormal metacercaria of *Alloglossidium corti*, showing the adult organs well developed.

Fig. 5.—Abnormal metacercaria of *Plagiorchis proximus*, with a few granules in the bladder and a beginning of the disintegration of the tail.

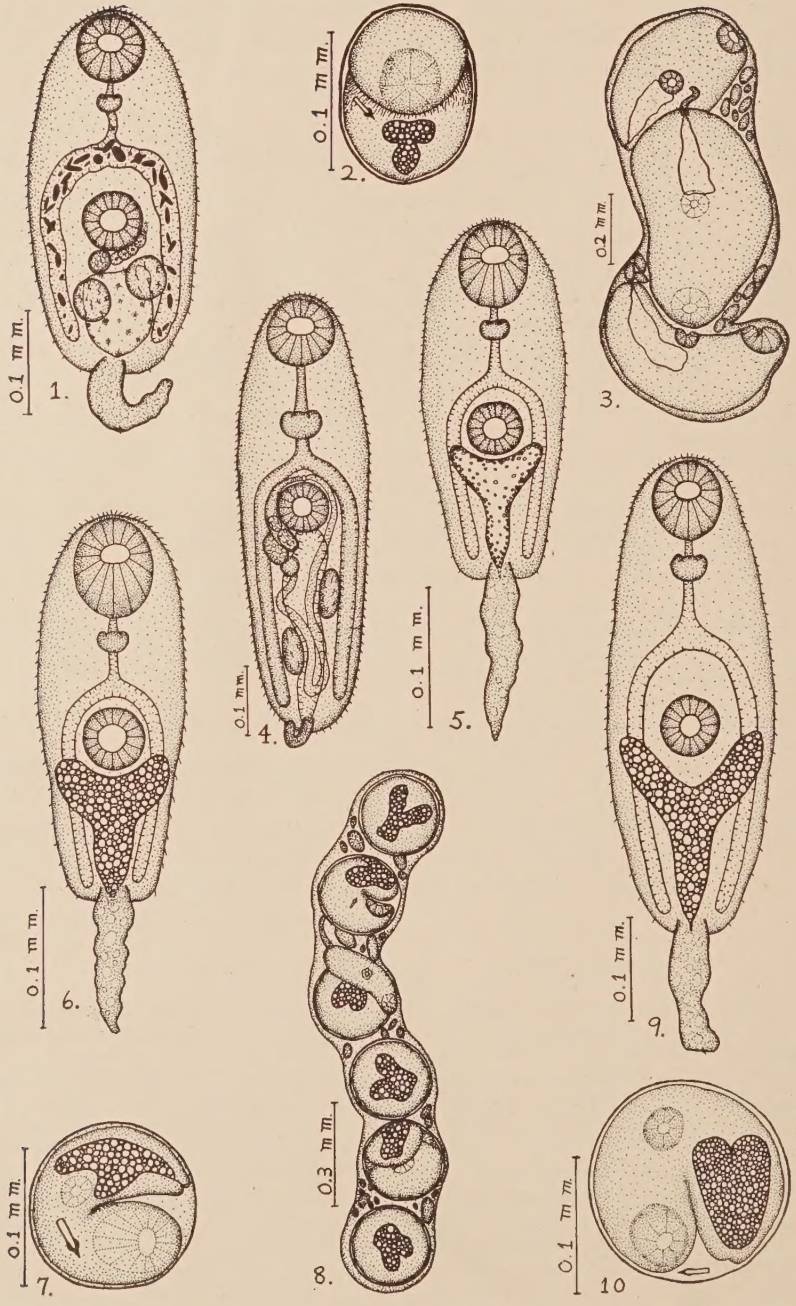
Fig. 6.—Abnormal metacercaria of *Plagiorchis proximus* in a more advanced stage.

Fig. 7.—Normal metacercaria of *Plagiorchis proximus*.

Fig. 8.—Normal metacercariae of *Plagiorchis muris* within the sporocyst.

Fig. 9.—Abnormal metacercaria of *Plagiorchis muris*.

Fig. 10.—Normal metacercaria of *Cercorchis medius*.


0.1 mm.
1.
0.1 mm.
2.
0.2 mm.
3.
0.1 mm.
4.
0.1 mm.
5.
0.1 mm.
6.
0.1 mm.
7.
0.3 mm.
8.
0.1 mm.
9.
0.1 mm.
10

RESEARCH NOTES

SUCKING LICE (ANOPLURA) ON MARMOTS

Recently Wilson (1937, Mammology, 18: 361–362) in a short article entitled "Lice on hibernating and non-hibernating mammals" stated that "No lice, sucking or biting, have ever been reported from a woodchuck." Inasmuch as the writer had only lately identified specimens of Anoplura in the parasite collection of the Division of Entomology and Economic Zoology, University of Minnesota, collected from *Marmota flaviventris nosophora* taken at Bridger Mountains, Montana, August 14, 1936, by Dr. W. A. Riley, as *Neohaematopinus marmotae* Ferris, 1923, it was deemed desirable that this record, along with others appearing in the literature, be reported. The specimens examined, about twenty-five in number, including both sexes, agree in all details with Ferris' description and figures.

Ferris in his catalogue of the Anoplura (1916, Proc. Calif. Acad. Sci., 4 ser., 6: 190) recorded *Linognathoides montanus* (Osborn, 1896) Kellogg and Ferris, 1915, from *Marmota flaviventris sierrae,* California and from *Marmota flaviventris* s.sp., Burns, Oregon, and *Linognathoides* sp. from *Marmota pruinosa* (=*Marmota caligata caligata*) (North America). More recenly Ferris (1919, Stanford Univ. Publ., Univ. Ser., Biol. Sc., 2: 47, fig. 30) described *Enderleinellus marmotae* (=*Cyclophthirus marmotae* (Ferris, 1919) Ewing, 1929) from *Marmota monax rufescens* taken at Grafton, South Dakota. He also reported them occurring on the same host from Elk River, Minnesota, and on *Marmota monax monax* from Marble Cave, Missouri, Sandy Springs, Maryland, and Washington, D. C. He later (Ferris, 1923, *ibid.,* 2: 268, figs. 171c, 171e, 171f) described *Neohaematopinus marmotae* (*Linognathoides montanus* (Osborn, 1896) in part, misidentification) taken from *Marmota flaviventris sierrae* at Yosemite National Park in California and from *Marmota* sp. at Florence, Montana. In addition, he stated that a single male specimen collected from *Marmota aurea,* Tagdumdash, Pamir, Asia (U. S. N. M. skin 62116), is doubtfully referred to this species.

In conclusion, it may be pointed out that since *Linognathoides* Cummings, 1914, is a synonym of *Neohaematopinus* Mjöberg, 1910 (Ferris, 1923, *ibid.,* 2: 237, 238), *Cyclophthirus marmotae* (Ferris, 1919) and *Neohaematopinus marmotae* Ferris, 1923, are the two species of Anoplura known to occur on marmots. —O. Wilford Olsen, *Division of Entomology and Economic Zoology, University of Minnesota, St. Paul. Paper No. 1539 of the Scientific Journal Series of the Minnesota Experiment Station.*

COPEPOD OBSERVED PREYING ON FIRST INSTAR LARVA OF *ANOPHELES QUADRIMACULATUS* SAY

Hinman (1934, J. Trop. Med. and Hyg., May 1) in a review of the literature on the predators of the larvae and pupae of the Culicidae found no reference to copepods as predators of these forms and a search of the literature since then has yielded no record of copepods in this rôle. It therefore seems worth while to record an observation made by the writer in the summer of 1937 while engaged in rearing out some larvae of *Anopheles quadrimaculatus* in the laboratory of the Tennessee Valley Authority at Wilson Dam, Alabama. A first instar larva which had hatched out in a Syracuse watch glass in which eggs had been placed for observation, was noticed struggling with an assailant of about equal side. On examining them under the microscope the predator was recognized as a copepod, later identified as *Cyclops* (*Microcyclops*) *varicans* by Dr. Charles B. Wilson, of Westfield, Mass. The crustacean had seized the larva at the posterior end and eventually severed the last two segments from the body, devouring them and discarding the long dorsal hairs of the anal segment.—Herbert S. Hurlbut, *Health Section, Tennessee Valley Authority, Wilson Dam, Ala.*

IN MEMORIAM

MAURICE CROWTHER HALL

July 15, 1881–May 1, 1938